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(54) Title: BARLEY WITH REDUCED SSII ACTIVITY AND STARCH CONTAINING PRODUCTS WITH A REDUCED AMYLOPECTIN CONTENT

(57) Abstract: Barley with reduced SSII activity has a starch structure with reduced amylopectin content and a consequent high relative amylose content. Additionally the grain has can have a relatively high β glucan content. The structure of the starch may also be altered in a number of ways which can be characterised by having a low gelatinsation temperature but with reduced swelling. The viscosity of gelatinised starch of the starch is also reduced. There is a chain length di... a low crystallinity of the starch. The starch is also characterised by having high levels of l... levels of V form starch crystallinity. The dietary fibre content of the starch is high. This ... characteristics.

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BARLEY WITH REDUCED SSII ACTIVITY AND STARCH AND STARCH CONTAINING PRODUCTS WITH A REDUCED AMYLOPECTIN CONTENT.

This invention relates to a barley plant with a reduced SSII enzyme activity leading to a starch having reduced amylopectin content. The invention also relates to starch and grain and food products obtained therefrom.

BACKGROUND OF THE INVENTION

One finding in nutritional science is that resistant starch has important implications for bowel health, in particular health of the large bowel. The beneficial effects of resistant starch result from the provision of a nutrient to the large bowel wherein the intestinal microflora are given an energy source which is fermented to form *inter alia* short chain fatty acids. These short chain fatty acids provide nutrients for the colonocytes, enhance the uptake of certain nutrients across the large bowel and promote physiological activity of the colon. Generally if resistant starches or other dietary fibre is not provided the colon is metabolically relatively inactive.

There has in recent years been a direction to look at providing for resistant starches from various sources to address bowel health. Accordingly high amylose starches have been developed in certain grains such as maize for use in foods as a means of promoting bowel health.

The physical structure of starch can have an important impact on the nutritional and handling properties of starch for food products. Certain characteristics can be taken as an indication of starch structure including the distribution of amylopectin chain length, the degree of crystallinity and the presence of forms of crystallinity such as the V-complex form of starch crystallinity. Forms of these characteristics can also be taken as indicator of nutritional or handling properties of foods containing these starches. Thus short amylopectin chain length may be an indicator of low crystallinity and low gelatinisation and is also thought to have a correlation with reduced retrogradation of amylopectin. Additionally shorter amylopectin chain length distribution is thought to reflect organoleptic properties of food in which the starch is included in significant amounts. Reduced crystallinity of a starch may also be indicative of a reduced gelatinisation temperature of starch and additionally it is thought to be associated with enhanced organoleptic properties. The presence of V-complex crystallinity or other starch associated lipid will enhance the level of resistant starch and thus dietary fibre.

of those foods or on the functional characteristics of those components in the preparation or structure of the foods.

Whilst modified starches or β glucans, for example, can be utilised in foods that provide functionality not normally afforded by unmodified sources, such processing has a tendency to either alter other components of value or carry the perception of being undesirable due to processes involved in modification. Therefore it is preferable to provide sources of constituents that can be used in unmodified form in foods.

The barley variety MK6827 is available from the Barley Germplasma Collection (USDA-ARS National Small Grain Germplasma Research Facility Aberdeen, Idaho 831290 USA). The grain of MK6827 is shrunken and has a highly coloured husk and an elongate shape and, in the hands of the inventors, this grain is very difficult to process including being very resistant to milling. The properties of MK6827 grain had not been characterised before, nor had the nature of the mutation been ascertained nor is it considered suitable for producing food.

SUMMARY OF THE INVENTION

This invention arises from the isolation and characterisation of SSII mutant of barley plants the grain of which is found to contain starch that has reduced amylopectin content and therefore high relative levels of amylose and therefore has elevated levels of dietary fibre.

The grain of the mutant and grain from crosses into certain genetic backgrounds additionally has an elevated level of β glucan. The combination of elevated β glucan level and resistant starch contributing to high dietary fibre is thought by the inventors to be unique to the present invention.

Additionally, at least in some genetic backgrounds, it is found that grain from such mutants contain starch that have high relative levels of amylose, and also have low gelatinisation temperatures. The low swelling charactistics of such starch during and following gelatinisation also has advantages in certain dietary and food processing applications.

Furthermore, grain from such mutants are found to contain starch that have high relative levels of amylose, the amylose levels found are higher than 50% of the starch content which is a level never before found in unmodified starch derived from barley.

by (+) yielded the Himalaya PCR pattern and lines denoted by (O) gave the 292 PCR result. Panel (A), the seed length to thickness ratio plotted against the percentage of starch chains with DP between 6 and 11; Panel (B) seed weight plotted against the percentage of starch chains with DP between 6 and 11

Figure 9 Sequence of a barley SSII cDNA (SEQ ID NO 1) from the cultivar Himalaya

Figure 10 The structure of the SSII genes from (1) *T. tauschii* (diploid wheat), (2) barley cultivar Morex. The thick lines represent exons and the thin lines introns. The straight line underneath each example indicates the region of the gene sequences. The dotted line represents a region of the barley SSII gene, from intron 7, that has not been sequenced but has been determined by PCR analysis to be approximately 3 kb in length.

Figure 11 Comparisons of the predicted SSII cDNAs from MK6827 (SEQ ID NO 2), Morex (SEQ ID NO 3) and 292 (SEQ ID NO 4), and a cDNA sequence of Himalaya (SEQ ID NO 1). Predicted sequences were generated by identifying regions of the genomic sequences present in the Himalaya SSII cDNA. The ATG start codon and wild type stop codon are indicated, as are additional stop codons present in MK6827 (#) and 292 (&) respectively.

Figure 12 Comparison of amino acid sequences deduced from the genes encoding SSII from barley lines 292 (SEQ ID NO 7), Morex (SEQ ID NO 5), MK6827 (SEQ ID NO 8), Himalaya (SEQ ID NO 8). Additional stop codons in 292 and MK6827 are indicated by the symbols (&) and (#) respectively.

Figure 13. Position of the mutations in MK6827 (SEQ ID NO 2) and 292 (SEQ ID NO 4) in the barley SSII gene.

Figure 14. Development and use of a PCR assay for the 292 mutation. (a) schematic representation of an SSII region from Himalaya amplified by the primers ZLSS2P4 and ZLBSSIIP5 (b) representation of the region amplified from the SSII gene from 292 using ZLSS2P4 and ZLBSSIIP5, showing the absence of one NlaIV site (c) agarose gel electrophoresis of NlaIV digested products from barley; Lane M; DNA marker ladder, lane 1: MK6827, lane 2; Himalaya; lane 3, Tantangara; lane 4, 292; lane 5, 342.

Figure 15. SDS-PAGE electrophoresis of starch granule proteins. Panel (A) 8% Acrylamide (37.5:1 Acryl/Bis) SDS-PAGE gel, electroblotted and probed with a SSII antibody produced against purified granule-bound SSII protein from Wheat. (B) 12.5% acrylamide (30:0.135 Acryl/Bis), silver stained. The migration of molecular weight standards of defined mass (units are kd) are indicated on each side of the figure.

Figure 16. A schematic representation of DNA constructs designed to down regulate SSII expression following stable transformation of barley (1) The SSII gene from nucleotides 1 to 2972 (see Figure 9 for sequence) is inserted between the promoter and terminator in the sense orientation. (2) The SSII gene is inserted between the promoter and terminator in the anti-sense orientation from nucleotides 2972 to 1 (see Figure 9 for sequence). (3) Duplex construct in which intron 3 of the barley SSII gene (between nucleotides 1559 and 2851) of the Morex SSII genomic sequence is inserted between exons 2 and 3 from the barley SSII cDNA from Himalaya (nucleotides 363 to 1157 from Figure 9).

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Glycaemic Index. Is a comparison of the effect of a test food such as white bread or glucose on excursions in blood glucose concentration. The Glycaemic Index is a measure of the likely

and resistant starch that does not, at least in broader forms of the invention require mixing of β glucan and soluble dietary fibre together or modification of the component parts.

To the best of the knowledge of the inventors the barley plant of the present invention is the first time that there has been a barley grain having elevated relative dietary fibre levels in the form of resistant starch having an elevated amylose level, that also has elevated levels of β glucan that are at the higher end of the typical levels of β glucan or that go beyond that level. Grains that have β glucan content that are still higher are of the waxy phenotype and therefore have low levels of amylose.

It is known that there is a wide variation in β glucan levels in barley in the range of about 4% to about 18% by weight of the barley, but more typically from 4% to about 8% (Izydorcyk *et al.*, (2000) *Journal of Agricultural and Food Chemistry* **48**, 982-989; Zheng *et al.*, (2000) *Cereal Chemistry* **77**, 140-144; Elfverson *et al.*, (1999) *Cereal Chemistry* **76**, 434-438; Andersson *et al.*, (1999) *Journal of the Science of Foods and Agriculture* **79**, 979-986; Oscarsson *et al.*, (1996) *J Cereal Science* **24**, 161-170; Fastnaught *et al.*, (1996) *Crop Science* **36**, 941-946). Enhanced barley strains have been developed, Prowashonupana for example, which have between about 15% and about 18% by weight β-glucan but has a waxy phenotype. This is sold commercially under the name Sustagrain™, (ConAgra™ Specially Grain Products Company, Omaha, Neb. USA).

The levels of β glucan contemplated by this invention may depend on the genetic background in which the amylopectin synthesis enzyme activity is reduced. However it is proposed that the reduction of the amylopectin synthesis activity will have the effect of elevating the relative level of dietary fibre which, in part, takes the form of amylose, and at the same time elevating the level of β glucan. One explanation for the concomitant elevation of β glucan with elevated relative amylose levels is that such elevation might be the result of a concentration effect of having reduced endosperm and may be further increased through the diversion of carbon from starch synthesis to β glucan synthesis.

Thus the grain of the barley plant preferably has a β glucan content that is greater than 6% of total non-hulled grain weight or more preferably greater than 7% and most preferably greater than 8%, however levels of β glucan in a waxy mutant has been measured as being as high as 15 to 18% and the present invention may contemplate levels as high, or higher, than that.

In a second preferable form the grain of the barley plant has a reduced gelatinsation temperature (as measured by differential scanning calorimetry)in addition to the relatively high amylose content. On the data shown for the exemplified barley this reduced gelatinisation temperature is not just reduced when compared to starch produced by barley with somewhat elevated amylose content but also when compared with starch produced from barley with starch having normal levels of amylose. Thus whilst the invention contemplates reduced gelatinisation temperatures relative to a corresponding high amylose starch, it may also contemplate a gelatinisation temperature reduced relative to that of starch with normal amylose levels.

Additionally in the genetic backgrounds thus far checked the starch is also characterised by a swelling in heated excess water that is lower than swelling of other starches tested.

In a third preferable form the starch has amylose levels of higher than 50% of the starch content which is a level never before found in unmodified starch derived from barley..

The starch of the present barley plant has a high relative amylose content and much higher than might be anticipated for a mutation in the SSII gene or other starch synthase gene. Thus in wheat mutants in SSII result in relative amylose levels of about 35% of starch. The amylose content of starch might be considered to be elevated when the content is significantly greater than the 25% or so that is present in normal barley grain and thus might be greater than about 30% w/w of total starch. Known barley plants considered to be high amylose have a content of 35-45%. The present invention however provides for barley with an amylose content that is greater than 50%, with is a level never before found in unmodified starch derived from barley.

The relative amylose content might be greater than 60% and more preferably, still greater than 70%. It may be desired to have even higher levels and thus it has been possible to achieve even higher levels in other plants by breeding with single mutations, such levels approach 90%. Thus the invention might encompass amylose levels of greater than 80% or greater than 90%.

It will be understood that the relative level of amylose referred to is in relation to total starch content, and thus the remainder of the starch might be predominantly of an intermediate type of starch or it might be predominantly amylopectin or a mixture of both. In the barley analysed the elevated level of amylose results from decreased amylopectin levels, and accordingly the relative level of amylose does not result from an increased synthesis of amylose.

It is known that β glucan has the effect of slowing digestion in the small intestine simply by its presence when together with another food component. Similarly it is known that resistant molecules that have close juxtaposition with starch granules help to mask the starch and contribute to its resistance by making it physically inaccessible. Elevated levels of amylose and other forms of starch as may arise from association with lipid will be further enhanced therefore by the presence and physical juxtaposition to the starch granules. Thus there is provided a significant enhancement of the effects of the resistant starch, as well as a provision of other beneficial effects arising from high β glucan levels.

Additionally it is known that there is a dose response in terms of the beneficial effects of resistant starch and β glucan. It is proposed therefore that the increased level of β glucan together with the increased levels of resistant starch will provide enhanced health benefits.

The combination of the levels of β glucan and resistant starch of at least preferred forms of this invention have not been found before and certainly not from one source without a degree of modification or purification and thus forms of the present invention provide for a single practical source of these benefits.

Another preferred aspect of the starch is that despite the high relative amylose content it also has a low gelatinisation temperature as measured by differential scanning calorimetry. This is in contrast with the general finding that high amylose starches tend to have a raised gelatinisation temperature which introduces restrictions on the manner in which high amylose starches can be utilised. On the data shown for the exemplified barley this reduced gelatinisation temperature is not just reduced when compared to starch produced by lines with somewhat elevated amylose content but also when compared with starch produced from barley with starch having normal levels of amylose. Thus whilst a preferred aspect of the invention contemplates reduced gelatinisation temperatures relative to corresponding high amylose starch it may also contemplate a gelatinisation temperature reduced relative to that of starch with

is about 3.75. Whereas the grains of the mutants and crosses examined are less than 3.2, preferably less than 3.0, but generally higher than about 2.

This low swelling gelatinisation characteristic is particularly useful where it is desired to increase the starch content of a food preparation, in particular a hydrated food preparation. In the present instance it might be desired to increase the dietary fibre content of a sol or other liquid preparation where there would otherwise be a restriction on delivery of the food preparation.

This characteristic in combination with the reduced gelatinisation temperature exhibited by the present starch provides a prospect of significantly enhancing the nutritional benefits of foods where there is a requirement of rapid preparation, such as instant soups and instant noodles.

It is postulated gelatinisation temperature effects are the result of an altered amylopectin structure in the endosperm of its grain, and one measurement of this structure is the distribution of chain lengths (degrees of polymerisation) of the starch molecules following debranching by isoamylase. An analysis of the chain length of the amylopectin content of the starch of the exemplified SSII mutants showed that when debranched they have a distribution of chain length in the range from 5 to 60 that is shorter than the distribution of starch yielded by non-mutant lines upon debranching. Starch with shorter chain lengths will also have a commensurate increase in frequency of branching. Thus the starch may also have a distribution of shorter amylopectin chain lengths. The proportion of starch chains that have a degree of polymerisation that falls in the range of 6 to 11 residues may be greater than 25%, more preferably greater than 30% and most preferably greater than 35%. The proportion of starch chains that have a degree of polymerisation that falls in the range of 12-30 residues may be less than 65%, more preferably less than 60% and most preferably less than about 55 %. The proportion of starch chains that have a degree of polymerisation that falls in the range of 31-60 residues may be less than about 10%, more preferably less than about 8% but also preferably greater than about 5% and more preferably greater than about 6%. Rather than taken individually combination of proportions of the three chain length ranges might be taken as an indicator that a starch is of a type that accords with the present invention.

grain. This form of starch is usually associated with retrograded starch, in particular where there has been contact with lipids. In the case of the present invention it is postulated that the structure of the starch permits the formation of an intimate relationship between plant lipids and starch which results in the V-complex structure. It is thought that this form of starch may have health benefits because it has reduced digestibility and therefore may contribute to resistant starch.

Other forms of structure can also result from lipid-starch interaction and include non crystalline lipid-starch complexes. Thus the invention might also be said to reside in a barley plant exhibiting appreciable amounts of starch-lipid complexes in the starch content of the endosperm of its grain resulting from reduced levels of activity of one or more amylopectin synthesis enzymes. Starches that contain starch lipid complexes, including those that exhibit V-complex structure, are also usually resistant to digestion and thus contribute to the dietary fibre levels. Preferably the proportion of crystalline starch exhibiting a form of crystallinity characteristic of a starch-lipid complex is greater than about 50% and more preferably greater than about 80%.

The starch additional to the presence of the V-complex form of starch may also exhibit no appreciable amounts of A complex forms of starch. Absence of A-complex might be taken as indicator of the presence of a starch of this invention.

It is also found that the pasting temperature of strchs and product made from the grain of thisinvention are considerably elevated. The pasting temperatures in known starches is less than 70°C, and this is for both normal and high amylose starches. The starches of the present invention however preferably exhibit pasting temperatures of higher than about 75°C or more preferably higher than about 80°C. It will be noted that these are empirical measures and might be taken as relative to those measurement of the other starches.

The starch of the exemplified barley plant is found to have significant amounts of dietary fibre and resistant starch, presumably this increase is at least in part as a result of the high relative level of amylose, however there may also be a contribution of dietary fibre by reason of starch/lipid complexes, including V-complex, or because of the intimate associate of amylose

monocotyledonous plants such as barley and for regeneration of plants from protoplasts or immature plant embryos are well known in the art, see for example, Canadian Patent Application 2092588 by Nehra, Australian Patent Application No 61781/94 by National Research Council of Canada, Australian Patent No 667939 by Japan Tobacco Inc., International Patent Application PCT/US97/10621 by Monsanto Company, US Patent 5589617, and other methods are set out in Patent specification WO99/14314.

Other known approaches to altering the activity of the amylopectin synthesis enzyme, other than the use of mutations may also be adopted. Thus, for example, this could be by expression of suitable antisense molecules that interfere with the transcription or processing of the gene or genes encoding the amylopectin synthesis enzyme. These might be based on the DNA sequence elucidated herein for the barley SSII gene. These antisense sequences can be for the structural genes or for sequences that effect control over the gene expression or splicing event. These sequences have been referred to above. Methods of devising antisense sequences are well known in the art and examples of these are can be found in, for example, United States Patent 5190131, European patent specification 0467349 AI, European patent specification 0223399 AI and European patent specification 0240208, which are incorporated herein by reference to the extent that they provide methods for carrying out antisense techniques. Methods of introducing and maintaining such sequences in plants are also published and known.

A variation of the antisense technique is to utilise ribozymes. Ribozymes are RNA molecules with enzymic function that can cleave other RNA molecules at specific sites defined by an antisense sequence. The cleavage of the RNA block the expression of the target gene. Reference is made to European patent specification 0321201 and specification WO 97/45545.

Another molecular biological approach that might also be used is that of co-suppression. The mechanism of co-suppression is not well understood, but it involves putting an extra copy of a gene into a plant in the normal orientation. In some instances the additional copy of the gene interferes with the expression of the target plant gene. Reference is made to Patent specification WO 97/20936 and European patent specification 0465572 for methods of implementing co-suppression approaches.

folate or antioxidants such as tocopherols and tocotrienols. Thus calcium is established in the provision of material for growth and deposition of bone and other calcified tissue and in lowering the risk of osteoporosis later in life. Folic acid is found to be protective against neural tube defects when consumed periconceptually and decreases the risk of cardiovascular disease thereby enhancing the effects of the combination of resistant starch and β-glucan. Folic acid also is thought to have an effect of lowering the risk of certain cancers. Tocopherol and tocotrienols carry the benefits of antioxidants and are believed to lower the risk of cancer and heart disease, and also have the effect of reducing the undesirable effects of oxidation of components of a food such as fatty acids which can result in rancidity. When these components of this preferred form of barley grain or products made therefrom constitute a convenient packaging with the one grain. One specific form of milled product might be one where the aleurone layer is included in the milled product. Particular milling process might be undertaken to enhance the amount of aleurone layer in the milled product. Such a method is referred to in Fenech *et al.*, ((1999) *J Nutr* **129**:1114-1119). Thus any product derived from grain milled or otherwise processed to include aleurone layer and germ will have the additional nutritional benefits, without the requirement of adding these elements from separate sources.

It will be understood that the barley plant of the present invention is preferably one having grain that is useful for food production and in particular for commercial food production. Such a production might include making of flour or other product that might be an ingredient in commercial food production. A lower level of usefulness might be a starch content greater than about 12% or perhaps greater than about 15%. Or similarly this might include the capacity to mill the grain; thus whilst pearled barley may be produced from most forms of grain certain configurations of grain are particularly resistant to milling. Another characteristic that might have an impact on a variety producing a commercially useable grain is discolouration of the product produced. Thus where the husk or other portion of the grain exhibits significant colouration, for example purple, this will come through with the product and limits its commercial applications to niche applications such as being a component of a bread containing coloured whole or kibbled grains. It is generally also more convenient that the barley plants are naked, because the presence of husks on barley grains introduces greater difficulty in processing the grain. Another aspect that might make a barley plant of higher value is on the basis of starch extraction from the grain, the higher extraction rates being more useful. Grain shape is also another feature the can impact on the commercial usefulness of a

might be desired to make double mutations in other barley mutants available with shrunken endosperms where the causal gene is not known.

In a further aspect the invention could be said to reside in the grain produced from a barley plant as referred to in this specification.

It will also be understood that the invention encompasses a processed grain including a milled, ground, kibbled, pearled or rolled grain or product obtained from the processed or whole grain of the barley plant referred to above, including flour. These products may be then used in various food products, for example farinaceous product such as breads, cakes biscuits and the like, or food additives, such as thickeners or to make malted or other barley drinks, noodles and quick soups.

Alternatively the invention encompasses starch isolated from the grain of the barley plant referred to above. Starch might be isolated by known techniques.

It will be understood that one benefit of the present invention is that it provides for one or more products that are of particular nutritional benefit, and moreover it does so without the need to modify the starch or other constituents of the barley grain.

However it may be desired to make modifications to the starch, β glucan or other constituent of the grain, and the invention encompasses such a modified constituent.

The method of modification are those known, and include the extraction of the starch or β glucan or other constituent by conventional methods and modification of the starches to for the desired resistant form.

Thus the starch or β glucan may be modified either singly of multiply though the use of a treatment selected from group including but not limited to, heat and/or moisture, physically (for example ball milling), enzymatically (using for example α or β amylase, pullalanase or the like), chemical hydrolysis (wet or dry using liquid or gaseous reagents), oxidation, cross bonding with difunctional reagents (for example sodium trimetaphosphate, phosphorous oxychloride), or carboxymethylation.

While it is clear that at least these four activities are required for normal starch granule synthesis in higher plants, multiple isoforms of each of the four activities are found in the endosperm of higher plants and specific roles have been proposed for individual isoforms on the basis of mutational analysis (Wang *et al,* 1998, Buleon *et al.*, 1998) or through the modification of gene expression levels using transgenic approaches (Abel *et al.*, 1996, Jobling *et al.*, 1999, Scwall *et al.*, 2000). However, the precise contributions of each isoform of each activity to starch biosynthesis are still not known, and it is not known whether these contributions differ markedly between species. In the cereal endosperm, two isoforms of ADPglucose pyrophosphorylase are present, one form within the amyloplast, and one form in the cytoplasm (Denyer *et al.*, 1996, Thorbjornsen *et al.*, 1996). Each form is composed of two subunit types. The shrunken (*sh2*) and brittle (*bt2*) mutants in maize represent lesions in large and small subunits respectively (Girouz and Hannah, 1994). Four classes of starch synthase are found in the cereal endosperm, an isoform exclusively localised within the starch granule, granule-bound starch synthase (GBSS), two forms that are partitioned between the granule and the soluble fraction (SSI, Li *et al.*, 1999a, SSII, Li *et al.*, 1999b) and a fourth form that is entirely located in the soluble fraction, SSIII (Cao *et al*, 2000, Li *et al.*, 1999b, Li *et al,* 2000). GBSS has been shown to be essential for amylose synthesis (Shure *et al.*, 1983), and mutations in SSII and SSIII have been shown to alter amylopectin structure (Gao *et al*, 1998, Craig *et al.*, 1998). No mutations defining a role for SSI activity have been described.

Three forms of branching enzyme are expressed in the cereal endosperm, branching enzyme I (BEI), branching enzyme IIa (BEIIa) and branching enzyme IIb (BEIIb) (Hedman and Boyer, 1982, Boyer and Preiss, 1978, Mizuno *et al.*, 1992, Sun *et al.*, 1997). In maize and rice, high amylose phenotypes have been shown to result from lesions in the BEIIb gene (Boyer and Preiss, 1981, Mizuno *et al.*, 1993). In these mutants, amylose content is significantly elevated, and the branch frequency of the residual amylopectin is reduced. In addition, there is a significant pool of material that is defined as "intermediate" between amylose and amylopectin (Boyer *et al.*, 1980, Takeda, *et al.*, 1993). Mutations defining the roles of BEIIa and BEI have yet to be described, although in potato down regulation of BEI alone causes minimal affects on starch structure (Filpse *et al.*, 1996). However, in potato the combination of down regulation of BEII and BEI provides a much higher amylose content than the down-regulation of BEII alone (Schwall *et al.*, 2000). Two types of debranching enzymes are present in higher plants and are defined on the basis of their substrate specificities, isoamylase

starch biosynthesis and illustrate how mutations in specific genes can have differing impacts on starch structure from one species to another.

Materials and Methods

Mutagenesis and Screening

The hull-less barley variety "Himalaya" was mutagenised using sodium azide according to Zwar and Chandler (1995). Selection of variants with altered grain morphology was carried out according to Green *et al.*, (1997). A total of 75 lines with shrunken endosperm phenotypes were identified and maintained according to Green *et al.*, (1997).

Starch Isolation

Starch was isolated from barley grain using the method of Schulman *et al.* (1991).

Methods for Amylose Determination

Determinations of the amylose/amylopectin ratio by an HPLC method for separating debranched starches, and an iodine binding method, were carried out as described by Batey and Curtin, (1996). Analysis of the amylose/amylopectin ratio by the analysis on non-debranched starches was carried out according to Case *et al.*, (1998).

Starch Content Measurement

Starch was determined using the total starch analysis kit supplied by Megazyme (Bray, Co Wicklow, Republic of Ireland).

Protein Content

Nitrogen was determined by the Kjeldahl method, and protein contents were calculated using a factor of 5.7.

β-Glucan Levels

β-Glucan was determined using the kit supplied by Megazyme (Bray, Co Wicklow, Republic of Ireland).

Starch Chain Length Distribution

Doubled haploids were produced from F1 plants derived from crosses between 292 and *Hordeum vulgare* cv Tantangara, and between 342 and *H. vulgare* cv Tantangara by Dr P. Davies, Waite Institute, Adelaide, Australia.

Linkage Analysis

Genetic linkage data was calculated using MapManager.

Construction of barley cDNA library

Five mgs of polyA+ mRNA from 10, 12 and 15 days post-anthesis of barley endosperm tissues was used for cDNA synthesis according to the protocols (Life Technology). The *Not*I-(dT)18 primer (Pharmacia Biotech) was used for the first stand of cDNA synthesis. The double strand cDNAs were ligated with a *Sal*I-*Xho*I adapter (Stratagene) and cloned to the *Sal*I-*Not*I arms of ZipLox (Life Technology) after digestion of cDNAs with *Not*I followed by size fractionation (SizeSep 400 spun Column of Pharmacia Biotech). The ligated cDNAs were packaged with Gigapack III Gold packaging extract (Stratagene). Titre of the library was 2×10^6 pfu tested with Y1090(ZL) strain of *E.coli*.

Cloning of specific cDNA regions of barley starch synthase II using PCR

The cDNA clone, wSSIIp1, was used for the screening of a cDNA library of barley. The cDNA clone, wSSIIp1 was generated by PCR using the primers ssIIa (TGTTGAGGTTCC ATGGCACGTTC SEQ.ID. NO 9) and ssIIb (AGTCGTTCTGCCGTATGATGTCG SEQ. ID. NO 10), amplifying the region between nucleotide positions 1,435 and 1,835 of wSSIIA (GenBank accession no: AF155217).

The amplification was performed using a FTS-1 thermal sequencer (Corbett, Australia) for 1 cycle of 95°C for 2 minutes; 35 cycles of 95°C for 30 seconds, 60°C for 1 minutes, 72°C for 2 minutes and 1 cycle of 25°C for 1 minute. The fragment wSSIIp1 was cloned into a pGEM-T vector (Promega)

Screening of barley cDNA library

A cDNA library, constructed from RNA from the endosperm of barley cv Himalaya, was screened with a 347-bp cDNA fragment, wSSIIp1 at the hybridisation conditions as previously described (Rahman *et al.*, 1998). Hybridisation was carried out in 50% formamide, 6 x SSPE, 0.5% SDS, 5 x Denhardt's and 1.7 µg/mL salmon sperm DNA at 42°C for 16 h, then washed 3 x with 2 x SSC containing 0.1% SDS at 65°C for 1 h per wash.

Doubled haploids were produced from F1 plants derived from crosses between 292 and *Hordeum vulgare* cv Tantangara, and between 342 and *H. vulgare* cv Tantangara by Dr P. Davies, Waite Institute, Adelaide, Australia.

Backcrossing Strategy

Crosses were made between 292 and *Hordeum vulgare* cv Sloop to generate F1 seed. Plants derived from the F1 seed were selfed to generate a population of F2 seed. The plants growing from these F2 seed were tested using a PCR assay and plants homozygous for the 292 mutation were backcrossed to Sloop (BC1). The F1 plants resulting from BC1 were again tested by PCR and plants heterozygous for the 292 mutation selected, and crossed back to Sloop (BC2). The F1 plants derived from BC2 were again analysed by PCR and plants heterozygous for the 292 mutation selected. These plants were either selfed to generate a BC2F2 population, or crossed again to Sloop (BC3). The F1 plants derived from BC3 were again analysed by PCR and plants heterozygous for the 292 mutation selected. These plants were selfed to generate a BC3F2 population. Plants derived from these seed were tested by PCR and plants homozygous for the 292 mutation selected for single seed descent and seed increase.

Results

Selection of Mutants

The identification of a range of mutants in the hull-less or naked barley variety "Himalaya" induced by a sodium azide treatment has been previously reported by Zwar and Chandler (1995). A group of 75 shrunken grain mutants were identified by the inventors and the amylose content of the starch from the shrunken seed was determined by HPLC (Figure 1). Two lines, 292 and 342, were found to have amylose contents of 71 and 62.5% respectively (Table 1). The amylose contents of 292 and 342 were substantially higher than the previously well characterised AC38 line (47% amylose, see Table 1). This study defines the genetic basis of the novel high amylose phenotype displayed by 292 and 342, and describes effects of the causal mutation on grain and starch structure and functionality.

<u>Grain Characteristics</u>

Grain size and morphology:

The effects of the mutation on grain weight and morphology are marked (Table 2). The grain weight is reduced from 51 mg for the parent line Himalaya, to 32 mg for 292 and 35 mg for

mg/caryopsis in 292 and 4.8 mg/caryopsis in 342. In contrast, there is a dramatic reduction in amylopectin synthesis per caryopsis, from 18.7 mg in Himalaya, to 1.6 mg in 292 and 2.9 mg in 342.

Chain Length Distribution

The chain length distribution of the starch following isoamylase debranching was carried out using fluorophore-assisted carbohydrate electrophoresis (FACE). The chain length distribution of the 292 and 342 mutants, and Himalaya, are shown in Figure 3a. Figure 3b shows a difference plot in which the normalised chain length distributions for the 292 and 342 mutants are subtracted from the normalised distribution of Himalaya. The percentages of chain lengths from DP 6-11, DP 12-30 and DP 31-65 have been calculated and are presented in Table 3. There is a marked shift in the 292 and 342 mutants in chain length distribution such that there is a higher percentage of chains in the region from DP6-11 compared to DP12-30.

Differential Scanning Calorimetry

The gelatinisation temperature of the mutants was investigated using differential scanning calorimetry, and the data is shown in Table 4. Both 292 and 342 yield starches that have markedly lower gelatinisation temperatures than the Himalaya starches, with respect to onset, peak and final temperatures for the gelatinisation peak. The enthalpy for the gelatinisation peak for the 292 and 342 mutants is also dramatically reduced in comparison to the wild type. The amylose/lipid peak onset temperature is also reduced for the 292 and 342 mutants, however, the enthalpy is increased, consistent with the increased amylose content of the mutants.

Starch Viscosity by RVA

RVA analysis of barley wholemeal samples was conducted in order to examine their pasting viscosity. Previous studies have shown that analysis of wholemeal samples is strongly correlated with the analysis of isolated starches (Batey *et al.*, 1997). The analysis showed that there are major differences between the barley genotypes studied (see Table 5 and Figure 4). Two barley varieties containing wild type starch, Himalaya and Namoi, showed typical RVA profiles in which there was a prominent peak viscosity, followed by a decline in viscosity to a holding strength, followed by an increase in viscosity as the temperature is reduced to a final viscosity. As is generally observed for barley starches, the final viscosities for the wild type starches were equivalent to, or less than, the peak viscosities (Table 5). In AC38, a prominent peak viscosity was obtained, however, because of the elevated amylose content of this line, the

Dietary Fibre

Dietary fibre analysis was conducted according to the AOAC procedure and showed that there was an increase in dietary fibre in 292 and 342, and that this increase in dietary fibre was due to an increase in insoluble dietary fibre rather than soluble dietary fibre (Table 1), consistent with components of the dietary fibre being resistant starch and β-glucan. It is to be noted that this measure of dietary fibre is a chemically determined one which is quite distinct form the physiological measure relevant from a nutritional point of view.

<u>Genetic Basis of the Mutation</u>

Segregation ratio

Crossing of the mutation to barley varieties not displaying the shrunken endosperm phenotype of 292 or 342 demonstrated that the mutation is a straightforward recessive mutation, displaying a 3 normal : 1 shrunken ratio in the F2 seed of outcrossed populations, and 1 normal : 1 shrunken ratio in the seed of a doubled haploid population developed following a single outcross (see Table 6). Normal is defined as seed with an L/T ratio of <3.5, shrunken seed as seed with an L/T ratio of >3.5.

Allelic nature of mutants

The 292 and 342 mutations were shown to be allelic through the analysis of progeny from crosses of 292 and 342. All F1 seed derived from reciprocal crosses showed grain weight and grain morphology phenotypes within the range of sizes and shapes observed for the parental 292 and 342 lines, and outside of the range of seed size and shape found for the parental Himalaya line. Furthermore, all F2 seed derived from 292 x 342 F1 plants showed the typical shrunken seed phenotype of the 292 and 342 mutants.

Analysis of the grain morphology and starch characteristics of a series of shrunken grain mutants available from the Barley Germplasm Collection (USDA-ARS, National Small Grains Germplasm Research Facility, Aberdeen, Idaho 83210, USA) suggested that the line MK6827 (BGS31, also referred to as GSHO 2476), carrying the *sex6* mutation showed a highly similar set of starch and grain characteristics to the 292 and 342 mutations. Crosses were established between 292 and MK6827 and all F1 grain showed the typical 292 phenotype with respect to grain weight and shrunken seed phenotype. F2 seed derived from the 292 x MK6827 F1 plants all showed shrunken endosperm phenotype with L/T ratios of >4. In contrast, F2 seed

mapped within 16.3 cM of the *nud* locus. This location is consistent with previous mapping data for the allelic *sex*6 mutation (Netsvetaev, 1990, Netsvetaev and Krestinkov, 1993, Biyashev *et al.*, 1986, Netsvetaev, 1992).

Identification of the causal gene

The *nud* gene has been demonstrated to be located on barley chromosome 7H (Figure 8, Fedak *et al.*, 1972). In wheat, three starch synthases (GBSS, SSI and SSII), and an isoamylase-type debranching enzyme (S. Rahman, personal communication) are located on the short arm of chromosome 7, the homologous chromosome (Yamamori and Endo, 1996, Li *et al.*, 1999a, Li *et al.*, 1999b, Li *et al.*, 2000). The close linkage to the *nud* locus suggested that the most probable candidate gene was the SSII gene. The wheat SSII gene has been cloned at the cDNA level (Li *et al*, 1999b; Genbank Accession No. AF155217) and at the genomic level (Li *et al.*, personal communication), and a barley cDNA has been isolated and cloned (Figure 9). The sequencing of barley and wheat SSII genomic sequences shows that the genes have very similar exon/intron structures, however, the lengths of the intron regions differ between sequences (Figure 10). Comparison of the Morex genomic sequence and the sequence of a cDNA from Himalaya (Figure 9) lead to the identification of deduced cDNA sequences from Morex, 292 and MK2827.

A G to A transition mutant was found in the SSII gene from 292 at a position that corresponds to 1829 of the alignment shown in Figure 11. This mutation introduces a stop codon into the 292 SSII open reading frame (Figure 12). Sequence analysis of Tantangara and Himalaya showed that both wild type genes were identical in this region and both 292 and 342 contained the same G to A transition mutation. The introduced stop codon would truncate the gene product such that the entire C-terminal catalytic domain of the starch synthase II gene would not be translated , and it is therefore highly likely that all SSII activity is abolished by this mutation.

A G to A transition was also present in MK6827, at position 242 of the alignment shown in Figure 11 and the Himalaya cDNA sequence in Figure 9. This mutation also introduces a stop codon into the 292 SSII open reading frame (Figure 12) and would prevent translation of over 90% of the SSII gene, abolishing SSII activity encoded by this gene.

DNA marker status is given in Table 8. More comprehensive analysis of the composition of these lines is given in Table 9, including RVA analysis, β-glucan content and flour swelling volume. The data shows that the lines carrying the 292 mutation have significantly different RVA parameters (as exemplified by the Peak/Final Viscosity ratio), higher β-glucan content, and altered flour swelling volumes.

In the second example, the mutation was transferred by performing two backcrosses from 292 to a cultivar with normal starch properties (cv Sloop). The F2 seed from three backcross 2 F1 plants was collected for analysis. The F2 seed were categorized into seed with an L:T ratio of >3.5 and an L:T ratio of <3.5. The distribution of seeds between these classes was consistent with expectations for a single recessive gene. Flour swelling volume data for the categories of seeds derived from each plant are shown in Figure 10 and shows that the starch swelling trait was clearly transferred through the breeding process into lines with an average of 75% Sloop background.

Discussion

We describe the isolation of novel mutants, 292 and 342, in barley that have a shrunken endosperm phenotype. Analysis of grain composition demonstrates that the shrunken phenotype is due to a significant decrease in starch content, and analysis of starch composition shows that this decrease is manifested as a high amylose phenotype that arises because of a decrease in amylopectin synthesis.

The 292 and 342 mutants possess a unique combination of grain and starch properties, in containing both increased β-glucan levels and resistant starch. The β-glucan levels of the lines are increased approximately 15% above that expected by the effect of reduced starch content, suggesting that carbon unable to be converted to starch is diverted to β-glucan synthesis. Determinations of dietary fibre levels demonstrate that the grain from the mutants have increased levels of dietary fibre, and that this increase is due to an increase in insoluble dietary fibre.

This combination of properties indicates that these mutants may have very interesting potential as components of the human diet. First, the elevated β-glucan levels suggests that the lines may be useful in lowering cholesterol through the well established action of β-glucan in reducing cholesterol levels. Secondly, the presence of resistant starch indicates that the lines

the crystal form shifts from the A type typical of cereal starches to a mixture of V and B types. The V type is typical of amylose and reflects the amylose component of the starch complexed with fatty acids, while the B form is derived from amylopectin and presumably reflects the residual amylopectin content of the starch (Buleon *et al*, 1998).

Analysis of the genetic basis of the 292 and 342 mutations demonstrates that the mutations are simple recessive mutations that give typical Mendelian ratios in outcrossing experiments. Crossing studies demonstrated that 292 and 342 are allelic. Further analysis of the interaction between 292 and other shrunken endosperm mutations in crossing experiments demonstrated that the 292/342 mutations were also allelic with the Sex6 mutation in the line MK6827. This mutation has previously been mapped and shown to be located within 3 cM of the centromere on the short arm of chromosome 7H (Netsvetaev, 1990, Netsvetaev and Krestinkov, 1993, Biyashev *et al.*, 1986, Netsvetaev, 1992).

A doubled haploid population between the husked barley Tantangara and the naked 292 mutant was established and the shrunken endosperm mutation mapped to the short arm of chromosome 7HS, to within 16 cM of the *nud* gene, a location consistent with the map location of the Sex6 mutation.

The localisation of the gene to the region adjacent to the centromere on the short arm of chromosome 7HS demonstrates that the causal mutation (sex6) is in a different gene to the mutation that causes the high amylose phenotype in AC38 (*amo1*) which has been mapped to chromosome 1H (Schondelmeier *et al* 1992). The map location suggested that one candidate for the gene disrupted in the sex6/292 mutation was starch synthase II, known in wheat to be localised in the same region of the chromosome (Yamamori and Endo 1996, Li *et al*, 1999b). Sequence analysis of the 292 and 342 mutants showed that there was a G to A transition mutation in the gene which would cause truncation of the gene such that the C-terminal region containing the active site of the enzyme would not be translated, presumably leading to the synthesis of a completely inactive protein. Furthermore, the sequencing of the SSII gene from MK6827 showed a G to A transition mutation at position 242 which would also cause truncation of the gene. This result confirms the allelic nature of the 292 and MK6827 mutations.

The availability of the sequence of the SSII gene and barley transformation systems provides the tools required to knock out the SSII gene using gene suppression technologies, in order to produce a comparable phenotype to that found with the SSII mutations. A recently developed highly effective strategy is to produce a hairpin construct designed to produce a double stranded RNA which would suppress the endogenous SSII activity. While complete knock out mutants analogous to the mutations described here would be of interest, the use of DNA constructs with differing promoters, and the recovery of transgenes with differing levels of hairpin construct expression, would allow the impact of titrating the expression of the gene from normal levels to complete knockdown levels to be assessed.

The mutations were shown to be able to be transferred from 292 into alternative barely genetic backgrounds, while retaining essential features of the original 292 mutation. In Tables 9 and 10, phenotypic data for 292 x Tantangara doubled haploid progeny, and the seed from a second backcross to Sloop, are shown, and indicate that the phenotypes are transferred through the breeding process.

Table 1
Barley Grain Composition

	Starch Content (%)[a]	Amylose Content By HPLC (%)[b]	Amylose Content by iodine binding (%)	Protein Content (%)[a]	β-glucan (%)[a]	Total Dietary Fibre [a] (%)	Insoluble Dietary Fibre [a] (%)	Soluble Dietary Fibre [a] (%)
Glacier	n.d.	31.0	n.d.	11.5	4.3	21.6	16.6	5
AC38	47	47.4	60.6	10.4	5.8	24.9	28.8	6.1
Himalaya	49	25	25.4	10.0	4.8	27.1	18.1	9
292	17.7	71	68.9	15.0	9.5	30.3	21.4	8.9
342	21.9	62.5	71.7	15.7	8.3	28.3	19.4	8.9
MK6827	10.2	n.d.	44.4	21.3	n.d.	n.d.	n.d.	n.d.
Waxiro	42.8	n.d.	5.0	14.6	n.d.	19.8	12.7	7.1.
Tantangara	51.6	n.d.	29.5	14.6	n.d.	17.2	12.7	4.5.

[a] % grain weight, 14% moisture
[b] % of total starch content
n.d. not determined

Table 4
Barley Starch Thermal Properties Measured by DSC

	Peak 1				Peak 2			
	Onset	Peak	End	ΔH	Onset	Peak	End	ΔH
Glacier	55.4	59.3	65.3	4.2	93.9	101.4	107.7	0.87
AC38	55.0	62.2	68.2	3.9	89.3	100.1	106.9	1.195
Himalaya	56.8	60.9	68.0	4.5	93.1	101.8	108.3	0.78
292	46.0	51.2	58.1	0.29	88.7	97.7	104.9	1.34
342	45.2	50.4	56.8	0.47	86.5	97.0	105.0	1.59

Table 5
RVA Parameters for Barley Starches

	Peak Viscosity	Breakdown	Holding Strength	Setback	Final Viscosity	Normalised Final Viscosity*	Pasting Temp (C)
Himalaya	871.5	653.1	218.4	235.8	454.2	926	64.9
Namoi	621.7	367.5	254.2	375.3	629.5	1284	65.9
AC38	226.7	87.3	139.4	188.4	327.8	697	68.9
292	92.1**	***	133.9	230	363.9	2055	89.5
342	110.9**	***	144.9	264.5	409.4	1869	87.9
MK6827	18.2**	***	25.7	43.3	69	676	n.d.

* Final viscosity divided by starch content of wholemeal
** Value registered at time of peak viscosity for Himalaya
*** Value was less than zero
n.d. not determined

Table 6
Starch Crystallinity Data

Sample	% H2O (W.B)	Crystallinity %*	A %*	B %*	V %*
292	29.6	9	-	13	87
342	35.8	12	-	18	81
AC38	26.1	19	93	7	(traces)
Himalaya	27.7	27	93	7	(traces)
Waxiro	29.7	41	94	6	-

(* _ ± 5%)

Table 7
Progeny Analysis

Table 8
Scoring of 292 x Tantangara Doubled Haploid Lines

Line Number[a]	Husk[b]	Seed Weight (mg)	L/T Ratio[c]	DP6-11 (%)[d]	Amylose Content[e]	PCR[f]
1	N	26	3.8	35.87	50.2	292
2	N	24	4.21	36.87	56.2	292
3	H	43	3.32	25.45	18.3	Wt
5	N	40	4.58	39.47	55.5	292
7	N	34	4.28	19.63	43.0	292
8	H	48	3.02	21.6	46.7	Wt
9	N	31	2.76	22.89	25.9	Wt
10	N	26	3.02	27.56	21.1	Wt
11	N	34	3.55	37.90	44.7	292
12	H	50	2.94	26.37	32.8	Wt
13	N	27	4.29	38.68	48.4	292
14	H	56	3.07	22.98	20.8	Wt
15	H	46	2.74	24.88	22.9	Wt
16	H	43	2.78	25.40	18.3	Wt
17	N	31	3.8	37.37	54.2	292
18	N	31	4.51	37.46	57.5	292
19	H	26	3.1	29.57	22.7	Wt
20	H	53	3.04	25.42	23.8	Wt
21	N	31	4.5	38.51	59.1	292
22	N	27	4.63	37.25	27.2	292
23	H	47	2.73	24.11	21.2	Wt
24	N	27	4.58	36.89	42.0	292
26	H	35	3.57	19.50	15.1	Wt
27	H	22	4.3	36.81	48.6	292
28	N	31	4.34	38.88	37.0	292
30	N	30	4.04	38.05	48.4	292
31	N	23	4.25	37.07	51.7	292
32	H	48	2.62	20.67	13.0	Wt
33	N	25	4.92	35.68	33.3	292
34	N	31	4.01	38.34	46.1	292
35	H	43	3.16	20.07	23.6	Wt
36	N	26	4.33	36.93	29.7	292
38	H	38	3.01	21.11	9.1	Wt
39	H	33	2.92	20.49	23.5	Wt
40	H	36	2.99	19.57	2.2	Wt
41	N	30	4.05	37.82	40.9	292
42	H	47	2.95	20.80	11.9	Wt
43	N	40	3.24	21.97	18.1	Wt
45	H	52	2.78	19.97	14.5	Wt
46	N	29	4.44	35.87	32.1	292
47	N	35	3.69	36.34	92.9	292
48	H	31	2.54	20.27	13.4	Wt
49	H	54	2.94	22.29	19.3	Wt
50	H	50	2.94	21.92	20.6	Wt

[c] L/T ratio: length to thickness ratio

[d] Percentage of chains in debranched starch with DP6 to DP11, calculated on a molar basis as a percentage of chains eluting between DP6 and DP65

[e]Amylose content determined by iodine blue value

[f] PCR score. 292, PCR reaction yields band which yields 169 bp band plus 103 bp on NlaIV digestion;Wt, PCR reaction yields band which yields 111 bp, 103 bp and 57 bp band on NlaIV digestion

Table 10
Flour Swelling Data for BC2F2 seed

Line	Swelling Volume
C5/1 Plant 1 L:T>3.5	2.118
C5/1 Plant 1 L:T<3.5	6.913
65/2 Plant 1 L:T>3.5	2.382
65/2 Plant 1 L:T<3.5	7.565
65/2 Plant 2 L:T>3.5	2.409
65/2 Plant 2 L:T<3.5	6.707

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CLAIMS

1. Starch obtained from starch granules of grain of a barley plant the barley plant having a reduced level of SSII activity, said starch granules having a high amylose content by reason of a reduced amylopectin content.

2. The starch of claim 1 with also exhibiting low gelatinisation temperatures.

3. The starch of claim 2 wherein the onset of the first peak detected by differential scanning calorimetry is reduced.

4. The starch of claim 2 wherein the first peak detected by differential scanning calorimetry is reduced.

5. The starch of claim 2 wherein the enthalpy (ΔH) of the first peak is reduced.

6. The starch of claim 2 exhibits a low swelling volume.

7. The starch of claim 6 having a swelling volume of less than about 3.2.

8. The starch of claim 6 having a swelling volume of less than about 3.0.

9. The starch of claim 6 having a swelling volume of higher than about 2.0.

10. The starch of claim 1 wherein the starch when gelatinised exhibits reduced peak viscosity.

11. The starch of claim 1 wherein the pasting temperature of the starch is higher than 80°C

12. The starch of claim 1 wherein the pasting temperature of the starch is higher than 75°C

13. The starch of claim 1 wherein amylose levels in the grain are higher than 30% (w/w) of the starch content.

25. The starch of claim 23 wherein the proportion of starch that exhibits crystallinity is less than about 20%

26. The starch of claim 1 wherein the starch exhibits reduced amylopectin chain length distribution

27. The starch of claim 26 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 6 to 11 residues is greater than 25%

28. The starch of claim 26 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 6 to 11 residues is greater than 30%

29. The starch of claim 26 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 6 to 11 residues is greater than 35%

30. The starch of claim 26 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 12-30 residues is less than 65%,

31. The starch of claim 26 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 12-30 residues is less than 60%,

32. The starch of claim 26 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 12-30 residues is less than 55%,

33. The starch of claim 26 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 31-60 residues is less than about 10%.

34. The starch of claim 26 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 31-60 residues is less than about 8%.

35. The starch of claim 34 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 31-60 residues is greater than about 5%.

36. The starch of claim 34 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 31-60 residues is greater than about 6%.

49. The grain of claim 48 wherein the pasting temperature of the starch is higher than 75°C

50. The grain of claim 49 wherein the pasting temperature of the starch is higher than 80°C

51. The grain of claim 37 wherein the grain has an elevated level of β glucan.

52. The grain of claim 51 wherein the β glucan content that is greater than 6% of total non-hulled grain weight

53. The grain of claim 51 wherein the β glucan content that is greater than 7% of total non-hulled grain weight

54. The grain of claim 51 wherein the β glucan content that is greater than 8% of total non-hulled grain weight

55. The grain of claim 51 wherein the β glucan content that is greater than about 15% of total non-hulled grain weight

56. The grain of claim 37 wherein the amylose content is higher than 30% (w/w) of the starch content.

57. The grain of claim 37 wherein the amylose content is higher than 50% (w/w) of the starch content

58. The grain of claim 37 wherein the amylose content is higher than 60% (w/w) of the starch content

59. The grain of claim 37 wherein the amylose content is higher than 70% (w/w) of the starch content

60. The grain of claim 37 exhibit appreciable amounts of starch associated lipid

72. The grain of claim 69 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 6 to 11 residues is greater than 35%.

73. The grain of claim 69 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 12-30 residues is less than 65%.

74. The grain of claim 69 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 12-30 residues is less than 60%.

75. The grain of claim 69 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 12-30 residues is less than 55%.

76. The grain of claim 69 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 31-60 residues is less than about 10%

77. The grain of claim 69 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 31-60 residues is less than about 8%

78. The grain of claim 77 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 31-60 residues is greater than about 5%,

79. The grain of claim 77 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 31-60 residues is greater than about 6%,

80. The grain of claim 37 in a state selected from the group comprising, milled, ground, pearled or rolled, kibbled, cracked, or whole grain.

81. The grain of claim 80 milled to enhance the amount of aleurone layer present.

82. The grain of claim 37 having a length to thickness ratio of 4 or less.

83. The grain of claim 37 having a length to thickness ratio of less than about 5.8

84. The grain of claim 37 having a length to thickness ratio of less than about 5.5.

98. The barely plant of claim 89 wherein the starch when gelatinised exhibits reduced peak viscosity.

99. The barley plant of claim 89 wherein the pasting temperature of the starch is elevated.

100. The barley plant of claim 99 wherein the pasting temperature of the starch is higher than 75°C

101. The barley plant of claim 99 wherein the pasting temperature of the starch is higher than 80°C

102. The barley plant of claim 89 wherein the grain has an elevated level of β glucan.

103. The barley plant of claim 102 wherein the β glucan content that is greater than 6% of total non-hulled grain weight

104. The barley plant of claim 102 wherein the β glucan content that is greater than 7% of total non-hulled grain weight

105. The barley plant of claim 102 wherein the β glucan content that is greater than 8% of total non-hulled grain weight

106. The barley plant of claim 102 wherein the β glucan content that is greater than about 15% of total non-hulled grain weight

107. The barley plant of claim 89 wherein the amylose content is higher than 30% (w/w) of the starch content.

108. The barley plant of claim 89 wherein the amylose content is higher than 50% (w/w) of the starch content

109. The barley plant of claim 89 wherein the amylose content is higher than 60% (w/w) of the starch content

122. The barley plant of claim 120 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 6 to 11 residues is greater than 30%.

123. The barley plant of claim 120 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 6 to 11 residues is greater than 35%.

124. The barley plant of claim 120 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 12-30 residues is less than 65%.

125. The barley plant of claim 120 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 12-30 residues is less than 60%.

126. The barley plant of claim 120 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 12-30 residues is less than 55%.

127. The barley plant of claim 120 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 31-60 residues is less than about 10%

128. The barley plant of claim 120 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 31-60 residues is less than about 8%

129. The barley plant of claim 128 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 31-60 residues is greater than about 5%,

130. The barley plant of claim 128 wherein the proportion of starch chains that have a degree of polymerisation that falls in the range of 31-60 residues is greater than about 6%,

131. The barley plant of claim 89 in a state selected from the group comprising, milled, ground, pearled or rolled, kibbled, cracked, or whole grain.

132. The barley plant of claim 131 milled to enhance the amount of aleurone layer present.

133. The barley plant of claim 89 having a length to thickness ratio of 4 or less.

134. The barley plant of claim 89 having a length to thickness ratio of less than about 5.8

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gccccatctg tcaatccaga acagagtacc ggtgaacggt gaaaacaaac ataaggtcgc 600
ctcgccgccg accagcatag tggatgtcgc gtctccgggt tccgcagcca acatttccat 660
cagtaacaag gtgccgccgt ccgttgtccc agccaagaag acgccgccgt cgtccgtttt 720
cccggccaag aaggcgccgc cgtcgtccgt tgtcccggcc aagaagacgc tgccgtcgtc 780
cggctcaaat tttgtgtcct cggcctctgc tcccaggctg gacactgtca gcgatgtgga 840
acttgcacag aagaaggatg cgctgattgt caaagaagct ccaaaaccaa aggctctttc 900
ggcccctgca gcccccgctg tacaagaaga ccctttggat ttcaagaaat acattggttt 960
cgaggagccc gtggaggcca aggatgatgg ctcggctgtt gcagatgatg cgggttcctt 1020
tgaacatcac cagaatcatg attccggacc tttggcaggg gagaacgtca tgaacgtggt 1080
cgtcgttgct gctgaatgtt ctccctggtg caaaacaggt ggtcttggag atgttgcggg 1140
tgctttgccc aaggctttgg ctaagagagg acatcgtgtt atggttgtgg taccaaggta 1200
tggggactat gaggaagcct acgatgtcgg agtccgaaaa tactacaagg ctgctggaca 1260
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caccatccct gtcgtgcacg ccgtcggcgg cctgagggat accgtgccgc cgttcgaccc 2340
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ggagcgcggc atgtcgcagg acttcagctg ggaacatgcc gccaagctct acgaggacgt 2520
cctcgtccag gccaagtacc agtggtgaac gctgctaccc ggtccagccc cgcatgcgtg 2580
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gtcgcagagt agagtgaaac gctccttgtt gcaggtatat gggaatgttt tttttccttt 2760
ttttttgcga gggaggtata tgggaatgtt aacttggtat tgtaatgtgg tatgctgtgt 2820
gcattattac atcggttgtt gttgcttatt cttgctagct aagtcggagg ccaagagcga 2880
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acggca 2946
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<210> 5
<211> 813
<212> PRT
<213> Hordeum vulgare
<220>
<223> Barley Cultivar Morex SSII deduced amino acid sequence
<400> 5

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                20                  25                  30
Ala Ser Pro Thr Arg Ala Gly Ala Gly Arg Leu Gln Trp Arg Pro
                35                  40                  45
Ser Pro Leu Gln Arg Thr Ala Arg Asp Gly Ala Val Ala Ala Arg
                50                  55                  60
Ala Ala Gly Ile Asp Asp Ala Ala Pro Gly Arg Gln Pro Arg Ala
                65                  70                  75
Arg Arg Tyr Gly Ala Ala Thr Lys Val Ala Asp Pro Val Lys Thr
                80                  85                  90
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Leu Asp Arg Asp Ala Ala Glu Gly Gly Gly Pro Ser Pro Pro Ala
95 100 105
Pro Arg Gln Asp Ala Ala Arg Leu Pro Ser Lys Asn Gly Thr Leu
110 115 120
Ile Asn Gly Glu Asn Lys Pro Thr Gly Gly Gly Gly Ala Thr Lys
125 130 135
Asp Ser Gly Leu Pro Thr Pro Ala Arg Ala Pro His Leu Ser Ile
140 145 150
Gln Asn Arg Val Pro Val Asn Gly Glu Asn Lys His Lys Val Ala
155 160 165
Ser Pro Pro Thr Ser Ile Val Asp Val Ala Ser Pro Gly Ser Ala
170 175 180
Ala Asn Ile Ser Ile Ser Asn Lys Val Pro Pro Ser Val Val Pro
185 190 195
Ala Lys Lys Thr Pro Pro Ser Ser Val Phe Pro Ala Lys Lys Ala
200 205 210
Pro Pro Ser Ser Val Val Pro Ala Lys Lys Thr Leu Pro Ser Ser
215 220 225
Gly Ser Asn Phe Val Ser Ser Ala Ser Ala Pro Arg Leu Asp Thr
230 235 240
Val Ser Asp Val Glu Leu Ala Gln Lys Lys Asp Ala Leu Ile Val
245 250 255
Lys Glu Ala Pro Lys Pro Lys Ala Leu Ser Ala Pro Ala Ala Pro
260 265 270
Ala Val Gln Glu Asp Leu Trp Asp Phe Lys Lys Tyr Ile Gly Phe
275 280 285
Glu Glu Pro Val Glu Ala Lys Asp Asp Gly Ser Ala Val Ala Asp
290 295 300
Asp Ala Gly Ser Phe Glu His His Gln Asn His Asp Ser Gly Pro
305 310 315
Leu Ala Gly Glu Asn Val Met Asn Val Val Val Val Ala Ala Glu
320 325 330
Cys Ser Pro Trp Cys Lys Thr Gly Gly Leu Gly Asp Val Ala Gly
335 340 345
Ala Leu Pro Lys Ala Leu Ala Lys Arg Gly His Arg Val Met Val
350 355 360
Val Val Pro Arg Tyr Gly Asp Tyr Glu Glu Ala Tyr Asp Val Gly
365 370 375
Val Arg Lys Tyr Tyr Lys Ala Ala Gly Gln Asp Met Glu Val Asn
380 385 390
Tyr Phe His Ala Tyr Ile Asp Gly Val Asp Phe Val Phe Ile Asp
395 400 405
Ala Pro Leu Phe Arg His Arg Gln Gln Asp Ile Tyr Gly Gly Ser
410 415 420
Arg Gln Glu Ile Met Lys Arg Met Ile Leu Phe Cys Lys Ala Ala
425 430 435
Val Glu Val Pro Trp His Val Pro Cys Gly Gly Val Pro Tyr Gly
440 445 450
Asp Gly Asn Leu Val Phe Ile Ala Asn Asp Trp His Thr Ala Leu
455 460 465
Leu Pro Val Tyr Leu Lys Ala Tyr Tyr Arg Asp His Gly Leu Met
470 475 480
Gln Tyr Ser Arg Ser Val Met Val Ile His Asn Ile Ala His Gln
485 490 495
Gly Arg Gly Pro Val Asp Glu Phe Pro Phe Thr Glu Leu Pro Glu
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His Tyr Leu Glu His Phe Arg Leu Tyr Asp Pro Val Gly Gly Glu
515 520 525
His Ala Asn Tyr Phe Ala Ala Gly Leu Lys Met Ala Asp Gln Val
530 535 540
Val Val Val Ser Pro Gly Tyr Leu Trp Glu Leu Lys Thr Val Glu
545 550 555
Gly Gly Trp Gly Leu His Asp Ile Ile Arg Gln Asn Asp Trp Lys
560 565 570

Thr Arg Gly Ile Val Asn Gly Ile Asp Asn Met Glu Trp Asn Pro
575 580 585
Glu Val Asp Val His Leu Lys Ser Asp Gly Tyr Thr Asn Phe Ser
590 595 600
Leu Lys Thr Leu Asp Ser Gly Lys Arg Gln Cys Lys Glu Ala Leu
605 610 615
Gln Arg Glu Leu Gly Leu Gln Val Arg Gly Asp Val Pro Leu Leu
620 625 630
Gly Phe Ile Gly Arg Leu Asp Gly Gln Lys Gly Val Glu Ile Ile
635 640 645
Ala Asp Ala Met Pro Trp Ile Val Ser Gln Asp Val Gln Leu Val
650 655 660
Met Leu Gly Thr Gly Arg His Asp Leu Glu Ser Met Leu Gln His
665 670 675
Phe Glu Arg Glu His His Asp Lys Val Arg Gly Trp Val Gly Phe
680 685 690
Ser Val Arg Leu Ala His Arg Ile Thr Ala Gly Ala Asp Ala Leu
695 700 705
Leu Met Pro Ser Arg Phe Glu Pro Cys Gly Leu Asn Gln Leu Tyr
710 715 720
Ala Met Ala Tyr Gly Thr Ile Pro Val Val His Ala Val Gly Gly
725 730 735
Leu Arg Asp Thr Val Pro Pro Phe Asp Pro Phe Asn His Ser Gly
740 745 750
Leu Gly Trp Thr Phe Asp Arg Ala Glu Ala His Lys Leu Ile Glu
755 760 765
Ala Leu Gly His Cys Leu Arg Thr Tyr Arg Asp His Lys Glu Ser
770 775 780
Trp Arg Gly Leu Gln Glu Arg Gly Met Ser Gln Asp Phe Ser Trp
785 790 795
Glu His Ala Ala Lys Leu Tyr Glu Asp Val Leu Val Gln Ala Lys
800 805 810
Tyr Gln Trp ***
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<211> 802
<212> PRT
<213> Hordeum vulgare
<220>
<223> Barley Cultivar Himalaya SSII deduced amino acid sequence
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Ala Ser Pro Thr Arg Ala Gly Ala Gly Arg Leu Gln Trp Arg Pro
35 40 45
Ser Pro Leu Gln Arg Thr Ala Arg Asp Gly Ala Val Ala Ala Arg
50 55 60
Ala Ala Gly Ile Asp Asp Ala Ala Pro Gly Arg Gln Pro Arg Ala
65 70 75
Arg Arg Tyr Gly Ala Ala Thr Lys Val Ala Asp Pro Val Lys Thr
80 85 90
Leu Asp Arg Asp Ala Ala Glu Gly Gly Gly Pro Ser Pro Pro Ala
95 100 105
Pro Arg Gln Asp Ala Ala Arg Leu Pro Ser Lys Asn Gly Thr Leu
110 115 120
Ile Asn Gly Glu Asn Lys Pro Thr Gly Gly Gly Gly Ala Thr Lys
125 130 135
Asp Ser Gly Leu Pro Thr Pro Ala Arg Ala Pro His Leu Ser Ile
140 145 150
Gln Asn Arg Val Pro Val Asn Gly Glu Asn Lys His Lys Val Ala
155 160 165
Ser Pro Pro Thr Ser Ile Val Asp Val Ala Ser Pro Gly Ser Ala
170 175 180

Ala Asn Ile Ser Ile Ser Asn Lys Val Pro Pro Ser Val Val Pro
185 190 195
Ala Lys Lys Thr Pro Pro Ser Ser Val Phe Pro Ala Lys Lys Thr
200 205 210
Leu Pro Ser Ser Gly Ser Asn Phe Val Ser Ser Ala Ser Ala Pro
215 220 225
Arg Leu Asp Thr Val Ser Asp Val Glu Leu Ala Gln Lys Lys Asp
230 235 240
Ala Leu Ile Val Lys Glu Ala Pro Lys Pro Lys Ala Leu Ser Ala
245 250 255
Pro Ala Ala Pro Ala Val Gln Glu Asp Leu Trp Asp Phe Lys Lys
260 265 270
Tyr Ile Gly Phe Glu Glu Pro Val Glu Ala Lys Asp Asp Gly Ser
275 280 285
Ala Val Ala Asp Asp Ala Gly Ser Phe Glu His His Gln Asn His
290 295 300
Asp Ser Gly Pro Leu Ala Gly Glu Asn Val Met Asn Val Val Val
305 310 315
Val Ala Ala Glu Cys Ser Pro Trp Cys Lys Thr Gly Gly Leu Gly
320 325 330
Asp Val Ala Gly Ala Leu Pro Lys Ala Leu Ala Lys Arg Gly His
335 340 345
Arg Val Met Val Val Val Pro Arg Tyr Gly Asp Tyr Glu Glu Ala
350 355 360
Tyr Asp Val Gly Val Arg Lys Tyr Tyr Lys Ala Ala Gly Gln Asp
365 370 375
Met Glu Val Asn Tyr Phe His Ala Tyr Ile Asp Gly Val Asp Phe
380 385 390
Val Phe Ile Asp Ala Pro Leu Phe Arg His Arg Gln Gln Asp Ile
395 400 405
Tyr Gly Gly Ser Arg Gln Glu Ile Met Lys Arg Met Ile Leu Phe
410 415 420
Cys Lys Ala Ala Val Glu Val Pro Trp His Val Pro Cys Gly Gly
425 430 435
Val Pro Tyr Gly Asp Gly Asn Leu Val Phe Ile Ala Asn Asp Trp
440 445 450
His Thr Ala Leu Leu Pro Val Tyr Leu Lys Ala Tyr Tyr Arg Asp
455 460 465
His Gly Leu Met Gln Tyr Ser Arg Ser Val Met Val Ile His Asn
470 475 480
Ile Ala His Gln Gly Arg Gly Pro Val Asp Glu Phe Pro Phe Thr
485 490 495
Glu Leu Pro Glu His Tyr Leu Glu His Phe Arg Leu Tyr Asp Pro
500 505 510
Val Gly Gly Glu His Ala Asn Tyr Phe Ala Ala Gly Leu Lys Met
515 520 525
Ala Asp Gln Val Val Val Val Ser Pro Gly Tyr Leu Trp Glu Leu
530 535 540
Lys Thr Val Glu Gly Gly Trp Gly Leu His Asp Ile Ile Arg Gln
545 550 555
Asn Asp Trp Lys Thr Arg Gly Ile Val Asn Gly Ile Asp Asn Met
560 565 570
Glu Trp Asn Pro Glu Val Asp Val His Leu Lys Ser Asp Gly Tyr
575 580 585
Thr Asn Phe Ser Leu Lys Thr Leu Asp Ser Gly Lys Arg Gln Cys
590 595 600
Lys Glu Ala Leu Gln Arg Glu Leu Gly Leu Gln Val Arg Gly Asp
605 610 615
Val Pro Leu Leu Gly Phe Ile Gly Arg Leu Asp Gly Gln Lys Gly
620 625 630
Val Glu Ile Ile Ala Asp Ala Met Pro Trp Ile Val Ser Gln Asp
635 640 645
Val Gln Leu Val Met Leu Gly Thr Gly Arg His Asp Leu Glu Ser
650 655 660

Met Leu Gln His Phe Glu Arg Glu His His Asp Lys Val Arg Gly
665 670 675
Trp Val Gly Phe Ser Val Arg Leu Ala His Arg Ile Thr Ala Gly
680 685 690
Ala Asp Ala Leu Leu Met Pro Ser Arg Phe Glu Pro Cys Gly Leu
695 700 705
Asn Gln Leu Tyr Ala Met Ala Tyr Gly Thr Ile Pro Val Val His
710 715 720
Ala Val Gly Gly Leu Arg Asp Thr Val Pro Pro Phe Asp Pro Phe
725 730 735
Asn His Ser Gly Leu Gly Trp Thr Phe Asp Arg Ala Glu Ala His
740 745 750
Lys Leu Ile Glu Ala Leu Gly His Cys Leu Arg Thr Tyr Arg Asp
755 760 765
His Lys Glu Ser Trp Arg Gly Leu Gln Glu Arg Gly Met Ser Gln
770 775 780
Asp Phe Ser Trp Glu His Ala Ala Lys Leu Tyr Glu Asp Val Leu
785 790 795
Val Gln Ala Lys Tyr Gln Trp ***
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<210> 7
<211> 813
<212> PRT
<213> Hordeum vulgare
<220>
<221> SITE
<222> 583
<223> Barley Line 292 SSII deduced amino acid sequence with premature termination
<400> 7

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Ala Ser Pro Thr Arg Ala Gly Ala Gly Arg Leu Gln Trp Arg Pro
35 40 45
Ser Pro Leu Gln Arg Thr Ala Arg Asp Gly Ala Val Ala Ala Arg
50 55 60
Ala Ala Gly Ile Asp Asp Ala Ala Pro Gly Arg Gln Pro Arg Ala
65 70 75
Arg Arg Tyr Gly Ala Ala Thr Lys Val Ala Asp Pro Val Lys Thr
80 85 90
Leu Asp Arg Asp Ala Ala Glu Gly Gly Gly Pro Ser Pro Pro Ala
95 100 105
Pro Arg Gln Asp Ala Ala Arg Leu Pro Ser Lys Asn Gly Thr Leu
110 115 120
Ile Asn Gly Glu Asn Lys Pro Thr Gly Gly Gly Gly Ala Thr Lys
125 130 135
Asp Ser Gly Leu Pro Thr Pro Ala Arg Ala Pro His Leu Ser Ile
140 145 150
Gln Asn Arg Val Pro Val Asn Gly Glu Asn Lys His Lys Val Ala
155 160 165
Ser Pro Pro Thr Ser Ile Val Asp Val Ala Ser Pro Gly Ser Ala
170 175 180
Ala Asn Ile Ser Ile Ser Asn Lys Val Pro Pro Ser Val Val Pro
185 190 195
Ala Lys Lys Thr Pro Pro Ser Ser Val Phe Pro Ala Lys Lys Ala
200 205 210
Pro Pro Ser Ser Val Val Pro Ala Lys Lys Thr Leu Pro Ser Ser
215 220 225
Gly Ser Asn Phe Val Ser Ser Ala Ser Ala Pro Arg Leu Asp Thr
230 235 240
Val Ser Asp Val Glu Leu Ala Gln Lys Lys Asp Ala Leu Ile Val
245 250 255
Lys Glu Ala Pro Lys Pro Lys Ala Leu Ser Ala Pro Ala Ala Pro

260 265 270
Ala Val Gln Glu Asp Leu Trp Asp Phe Lys Lys Tyr Ile Gly Phe
275 280 285
Glu Glu Pro Val Glu Ala Lys Asp Asp Gly Ser Ala Val Ala Asp
290 295 300
Asp Ala Gly Ser Phe Glu His His Gln Asn His Asp Ser Gly Pro
305 310 315
Leu Ala Gly Glu Asn Val Met Asn Val Val Val Val Ala Ala Glu
320 325 330
Cys Ser Pro Trp Cys Lys Thr Gly Gly Leu Gly Asp Val Ala Gly
335 340 345
Ala Leu Pro Lys Ala Leu Ala Lys Arg Gly His Arg Val Met Val
350 355 360
Val Val Pro Arg Tyr Gly Asp Tyr Glu Glu Ala Tyr Asp Val Gly
365 370 375
Val Arg Lys Tyr Tyr Lys Ala Ala Gly Gln Asp Met Glu Val Asn
380 385 390
Tyr Phe His Ala Tyr Ile Asp Gly Val Asp Phe Val Phe Ile Asp
395 400 405
Ala Pro Leu Phe Arg His Arg Gln Gln Asp Ile Tyr Gly Gly Ser
410 415 420
Arg Gln Glu Ile Met Lys Arg Met Ile Leu Phe Cys Lys Ala Ala
425 430 435
Val Glu Val Pro Trp His Val Pro Cys Gly Gly Val Pro Tyr Gly
440 445 450
Asp Gly Asn Leu Val Phe Ile Ala Asn Asp Trp His Thr Ala Leu
455 460 465
Leu Pro Val Tyr Leu Lys Ala Tyr Tyr Arg Asp His Gly Leu Met
470 475 480
Gln Tyr Ser Arg Ser Val Met Val Ile His Asn Ile Ala His Gln
485 490 495
Gly Arg Gly Pro Val Asp Glu Phe Pro Phe Thr Glu Leu Pro Glu
500 505 510
His Tyr Leu Glu His Phe Arg Leu Tyr Asp Pro Val Gly Gly Glu
515 520 525
His Ala Asn Tyr Phe Ala Ala Gly Leu Lys Met Ala Asp Gln Val
530 535 540
Val Val Val Ser Pro Gly Tyr Leu Trp Glu Leu Lys Thr Val Glu
545 550 555
Gly Gly Trp Gly Leu His Asp Ile Ile Arg Gln Asn Asp Trp Lys
560 565 570
Thr Arg Gly Ile Val Asn Gly Ile Asp Asn Met Glu *** Asn Pro
575 580 585
Glu Val Asp Val His Leu Lys Ser Asp Gly Tyr Thr Asn Phe Ser
590 595 600
Leu Lys Thr Leu Asp Ser Gly Lys Arg Gln Cys Lys Glu Ala Leu
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Gln Arg Glu Leu Gly Leu Gln Val Arg Gly Asp Val Pro Leu Leu
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Gly Phe Ile Gly Arg Leu Asp Gly Gln Lys Gly Val Glu Ile Ile
635 640 645
Ala Asp Ala Met Pro Trp Ile Val Ser Gln Asp Val Gln Leu Val
650 655 660
Met Leu Gly Thr Gly Arg His Asp Leu Glu Ser Met Leu Gln His
665 670 675
Phe Glu Arg Glu His His Asp Lys Val Arg Gly Trp Val Gly Phe
680 685 690
Ser Val Arg Leu Ala His Arg Ile Thr Ala Gly Ala Asp Ala Leu
695 700 705
Leu Met Pro Ser Arg Phe Glu Pro Cys Gly Leu Asn Gln Leu Tyr
710 715 720
Ala Met Ala Tyr Gly Thr Ile Pro Val Val His Ala Val Gly Gly
725 730 735
Leu Arg Asp Thr Val Pro Pro Phe Asp Pro Phe Asn His Ser Gly

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                    740                     745                     750
Leu Gly Trp Thr Phe Asp Arg Ala Glu Ala His Lys Leu Ile Glu
                    755                     760                     765
Ala Leu Gly His Cys Leu Arg Thr Tyr Arg Asp His Lys Glu Ser
                    770                     775                     780
Trp Arg Gly Leu Gln Glu Arg Gly Met Ser Gln Asp Phe Ser Trp
                    785                     790                     795
Glu His Ala Ala Lys Leu Tyr Glu Asp Val Leu Val Gln Ala Lys
                    800                     805                     810
Tyr Gln Trp ***
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<211> 813
<212> PRT
<213> Hordeum vulgare
<220>
<221> SITE
<222> 43
<223> Barley Line MK6827 SSII deduced amino acid sequence with premature
termination
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                35                  40                      45
Ser Pro Leu Gln Arg Thr Ala Arg Asp Gly Ala Val Ala Ala Arg
                50                  55                      60
Ala Ala Gly Ile Asp Asp Ala Ala Pro Gly Arg Gln Pro Arg Ala
                65                  70                      75
Arg Arg Tyr Gly Ala Ala Thr Lys Val Ala Asp Pro Val Lys Thr
                80                  85                      90
Leu Asp Arg Asp Ala Ala Glu Gly Gly Gly Pro Ser Pro Pro Ala
                95                  100                     105
Pro Arg Gln Asp Ala Ala Arg Leu Pro Ser Lys Asn Gly Thr Leu
                110                 115                     120
Ile Asn Gly Glu Asn Lys Pro Thr Gly Gly Gly Gly Ala Thr Lys
                125                 130                     135
Asp Ser Gly Leu Pro Thr Pro Ala Arg Ala Pro His Leu Ser Ile
                140                 145                     150
Gln Asn Arg Val Pro Val Asn Gly Glu Asn Lys His Lys Val Ala
                155                 160                     165
Ser Pro Pro Thr Ser Ile Val Asp Val Ala Ser Pro Gly Ser Ala
                170                 175                     180
Ala Asn Ile Ser Ile Ser Asn Lys Val Pro Pro Ser Val Val Pro
                185                 190                     195
Ala Lys Lys Thr Pro Pro Ser Ser Val Phe Pro Ala Lys Lys Ala
                200                 205                     210
Pro Pro Ser Ser Val Val Pro Ala Lys Lys Thr Leu Pro Ser Ser
                215                 220                     225
Gly Ser Asn Phe Val Ser Ser Ala Ser Ala Pro Arg Leu Asp Thr
                230                 235                     240
Val Ser Asp Val Glu Leu Ala Gln Lys Lys Asp Ala Leu Ile Val
                245                 250                     255
Lys Glu Ala Pro Lys Pro Lys Ala Leu Ser Ala Pro Ala Ala Pro
                260                 265                     270
Ala Val Gln Glu Asp Leu Trp Asp Phe Lys Lys Tyr Ile Gly Phe
                275                 280                     285
Glu Glu Pro Val Glu Ala Lys Asp Asp Gly Ser Ala Val Ala Asp
                290                 295                     300
Asp Ala Gly Ser Phe Glu His His Gln Asn His Asp Ser Gly Pro
                305                 310                     315
Leu Ala Gly Glu Asn Val Met Asn Val Val Val Val Ala Ala Glu
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Cys Ser Pro Trp Cys Lys Thr Gly Gly Leu Gly Asp Ile Ala Gly
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Ala Leu Pro Lys Ala Leu Ala Lys Arg Gly His Arg Val Met Val
                350                 355                 360
Val Val Pro Arg Tyr Gly Asp Tyr Glu Glu Ala Tyr Asp Val Gly
                365                 370                 375
Val Arg Lys Tyr Tyr Lys Ala Ala Gly Gln Asp Met Glu Val Asn
                380                 385                 390
Tyr Phe His Ala Tyr Ile Asp Gly Val Asp Phe Val Phe Ile Asp
                395                 400                 405
Ala Pro Leu Phe Arg His Arg Gln Gln Asp Ile Tyr Gly Gly Ser
                410                 415                 420
Arg Gln Glu Ile Met Lys Arg Met Ile Leu Phe Cys Lys Ala Ala
                425                 430                 435
Val Glu Val Pro Trp His Val Pro Cys Gly Gly Val Pro Tyr Gly
                440                 445                 450
Asp Gly Asn Leu Val Phe Ile Ala Asn Asp Trp His Thr Ala Leu
                455                 460                 465
Leu Pro Val Tyr Leu Lys Ala Tyr Tyr Arg Asp His Gly Leu Met
                470                 475                 480
Gln Tyr Ser Arg Ser Val Met Val Ile His Asn Ile Ala His Gln
                485                 490                 495
Gly Arg Gly Pro Val Asp Glu Phe Pro Phe Thr Glu Leu Pro Glu
                500                 505                 510
His Tyr Leu Glu His Phe Arg Leu Tyr Asp Pro Val Gly Gly Glu
                515                 520                 525
His Ala Asn Tyr Phe Ala Ala Gly Leu Lys Met Ala Asp Gln Val
                530                 535                 540
Val Val Val Ser Pro Gly Tyr Leu Trp Glu Leu Lys Thr Val Glu
                545                 550                 555
Gly Gly Trp Gly Leu His Asp Ile Ile Arg Gln Asn Asp Trp Lys
                560                 565                 570
Thr Arg Gly Ile Val Asn Gly Ile Asp Asn Met Glu Trp Asn Pro
                575                 580                 585
Glu Val Asp Val His Leu Lys Ser Asp Gly Tyr Thr Asn Phe Ser
                590                 595                 600
Leu Lys Thr Leu Asp Ser Gly Lys Arg Gln Cys Lys Glu Ala Leu
                605                 610                 615
Gln Arg Glu Leu Gly Leu Gln Val Arg Gly Asp Val Pro Leu Leu
                620                 625                 630
Gly Phe Ile Gly Arg Leu Asp Gly Gln Lys Gly Val Glu Ile Ile
                635                 640                 645
Ala Asp Ala Met Pro Trp Ile Val Ser Gln Asp Val Gln Leu Val
                650                 655                 660
Met Leu Gly Thr Gly Arg His Asp Leu Glu Ser Met Leu Gln His
                665                 670                 675
Phe Glu Arg Glu His His Asp Lys Val Arg Gly Trp Val Gly Phe
                680                 685                 690
Ser Val Arg Leu Ala His Arg Ile Thr Ala Gly Ala Asp Ala Leu
                695                 700                 705
Leu Met Pro Ser Arg Phe Glu Pro Cys Gly Leu Asn Gln Leu Tyr
                710                 715                 720
Ala Met Ala Tyr Gly Thr Ile Pro Val Val His Ala Val Gly Gly
                725                 730                 735
Leu Arg Asp Thr Val Pro Pro Phe Asp Pro Phe Asn His Ser Gly
                740                 745                 750
Leu Gly Trp Thr Phe Asp Arg Ala Glu Ala His Lys Leu Ile Glu
                755                 760                 765
Ala Leu Gly His Cys Leu Arg Thr Tyr Arg Asp His Lys Glu Ser
                770                 775                 780
Trp Arg Gly Leu Gln Glu Arg Gly Met Ser Gln Asp Phe Ser Trp
                785                 790                 795
Glu His Ala Ala Lys Leu Tyr Glu Asp Val Leu Val Gln Ala Lys
                800                 805                 810
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Tyr Gln Trp ***
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<213> Artificial
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<210> 10
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<223> PCR primer ssIIb
<400> 10
agtcgttctg ccgtatgatg tcg 23
<210> 11
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<223> PCR primer ZLSS2P4
<400> 11
cctggaacac ttcagactgt acg 23
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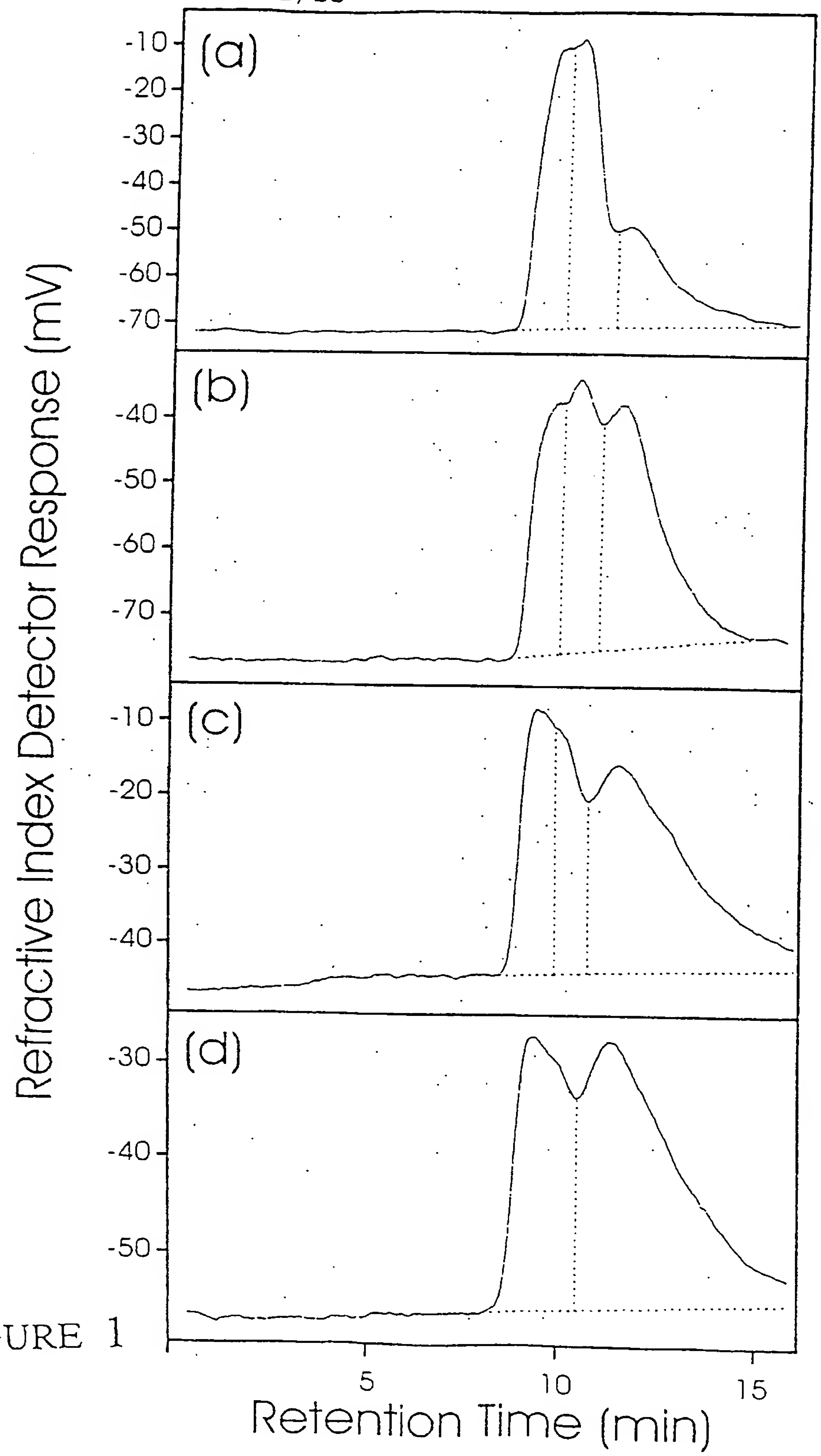


(a)
(b)
(c)
(d)
-10
-20
-30
-40
-50
-60
-70
Refractive Index Detector Response (mV)
5
10
15
Retention Time (min)

FIGURE 1

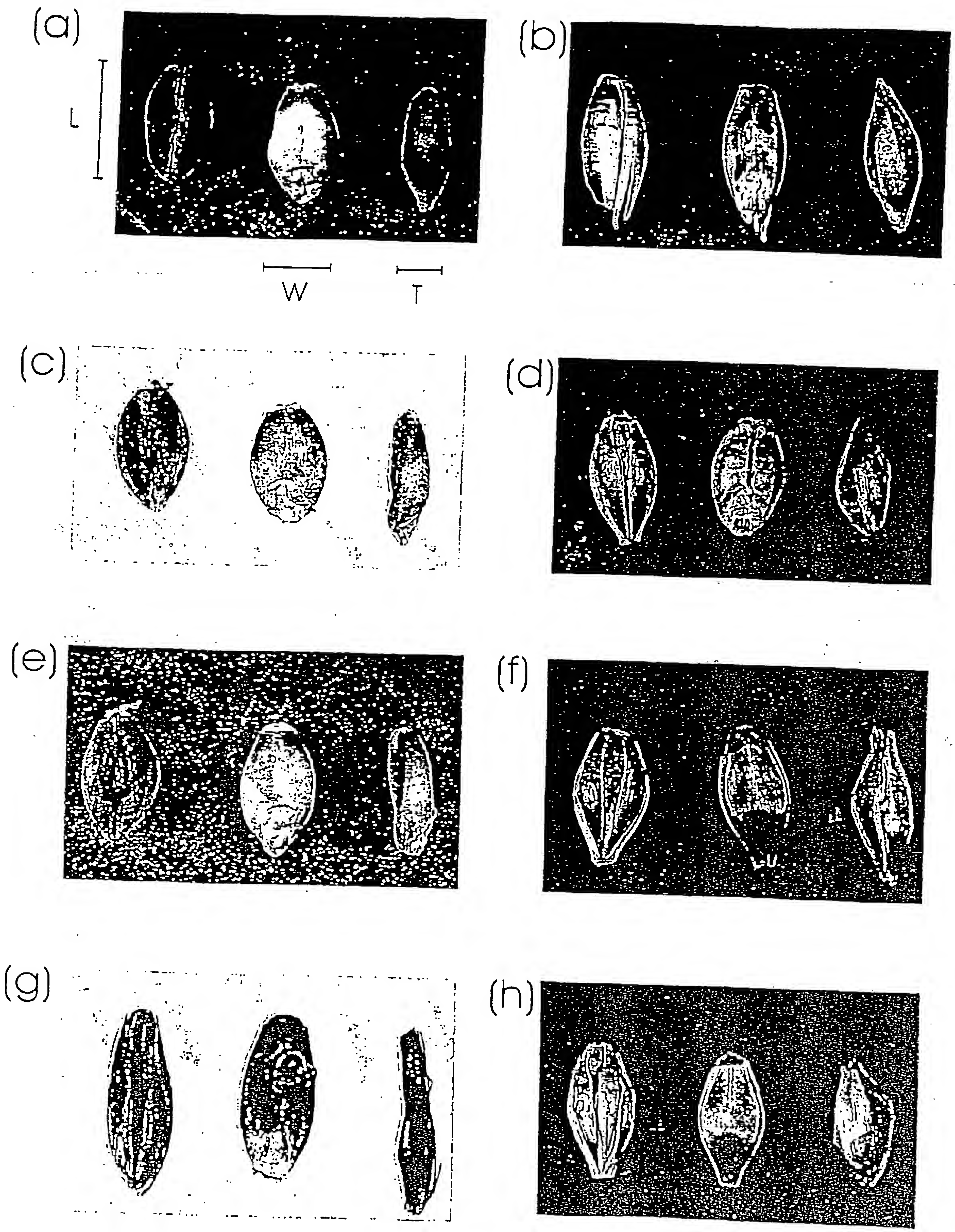


FIGURE 2

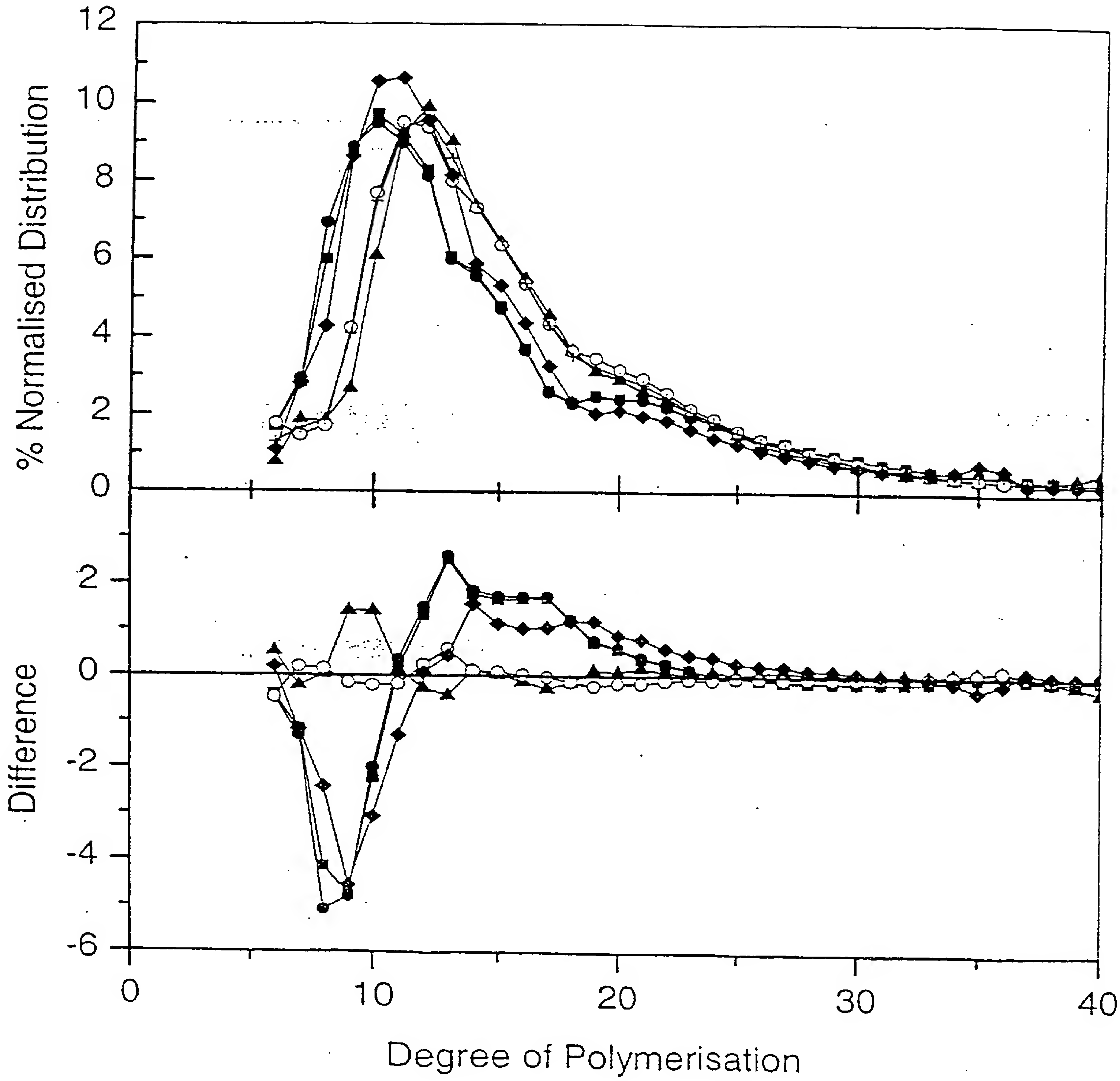
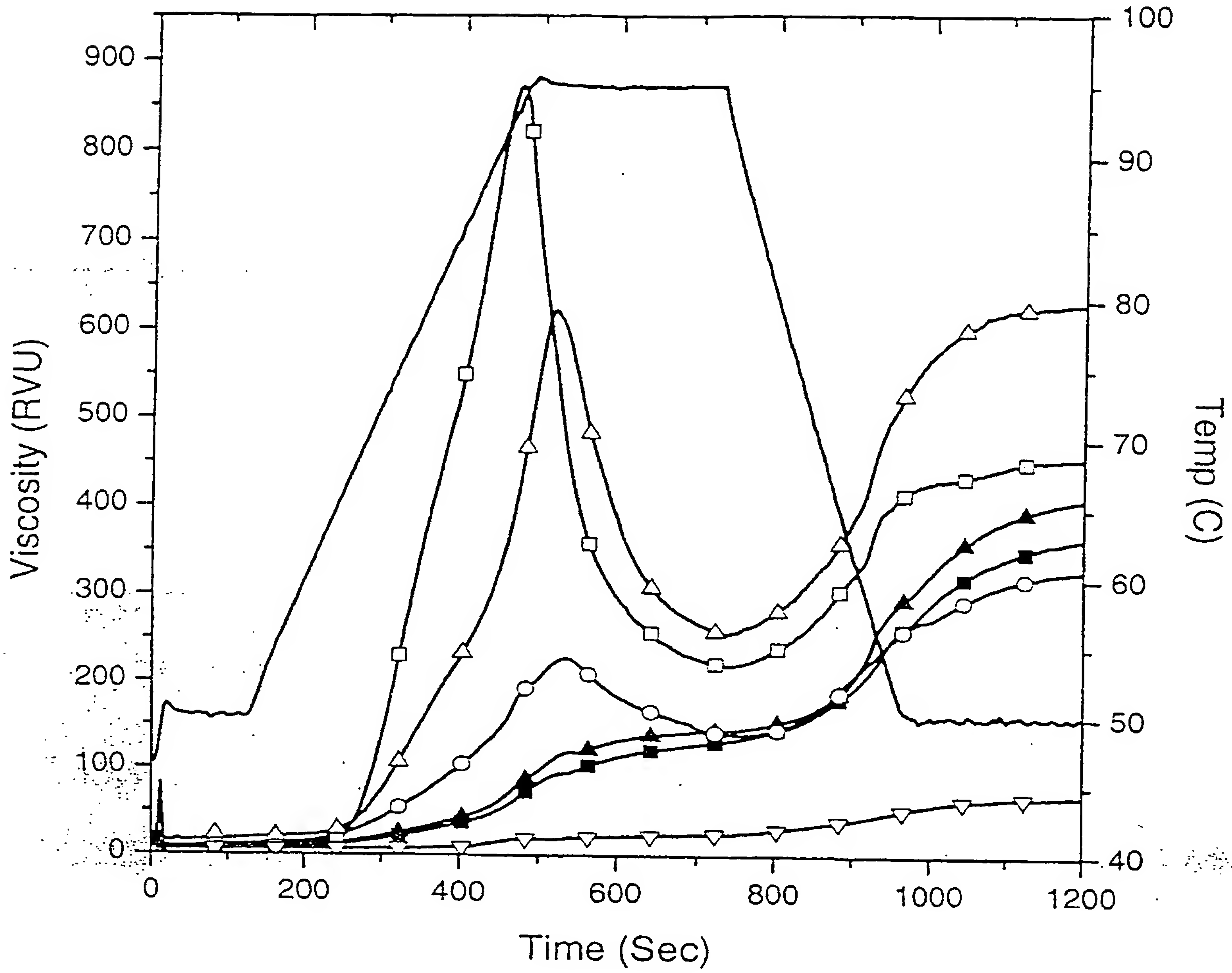

12
10
8
6
4
2
0
% Normalised Distribution
2
0
-2
-4
-6
Difference
0
10
20
30
40
Degree of Polymerisation

FIGURE 3



900
800
700
600
500
400
300
200
100
0
Viscosity (RVU)
100
90
80
70
60
50
40
Temp (C)
0
200
400
600
800
1000
1200
Time (Sec)

FIGURE 4

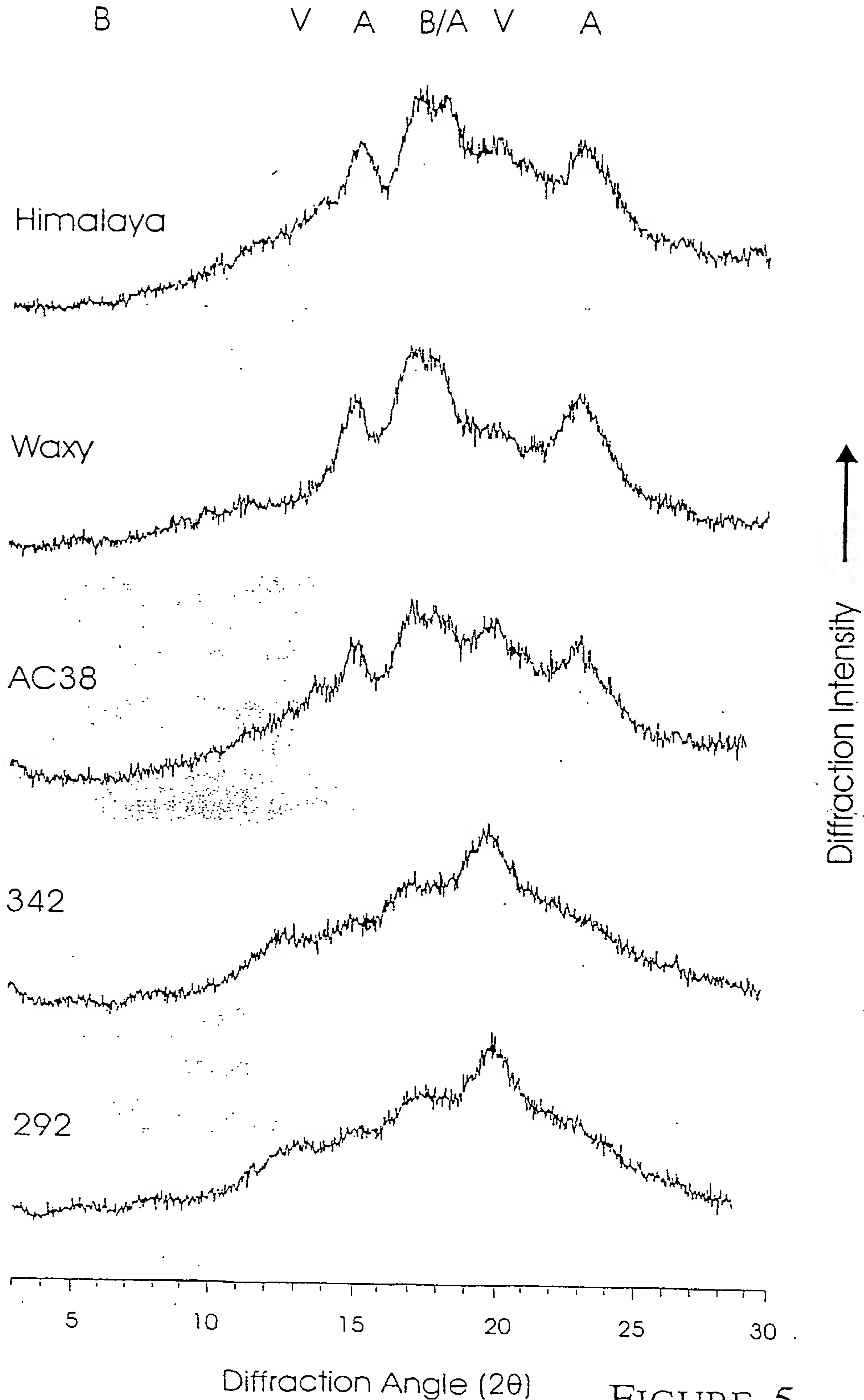

B
V
A
B/A
V
A
Himalaya
Waxy
AC38
342
292
Diffraction Intensity
5
10
15
20
25
30
Diffraction Angle (2θ)

FIGURE 5

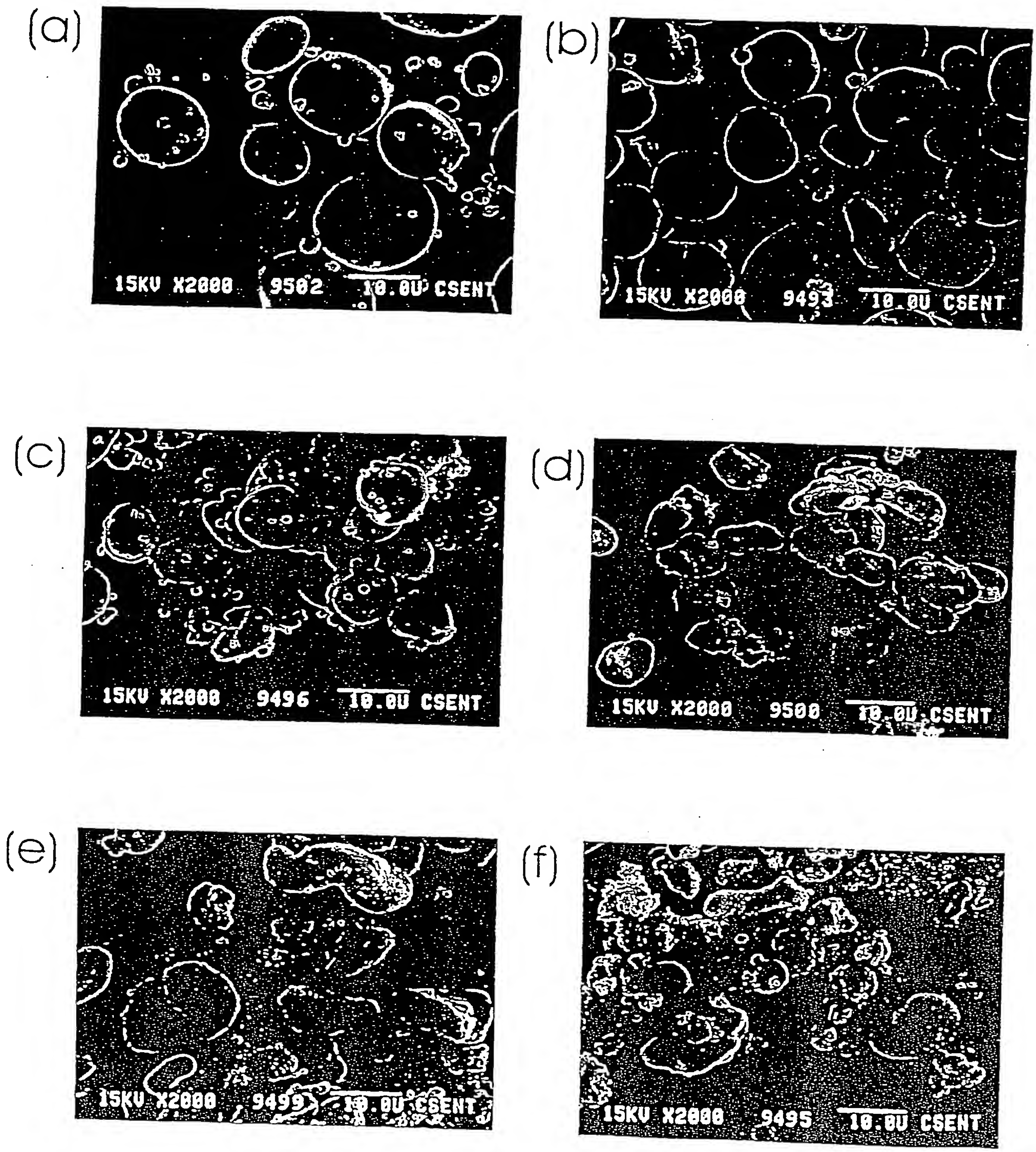

(a)
15KV X2000 9502 10.0U CSENT
(b)
15KV X2000 9493 10.0U CSENT
(c)
15KV X2000 9496 10.0U CSENT
(d)
15KV X2000 9500 10.0U CSENT
(e)
15KV X2000 9499 10.0U CSENT
(f)
15KV X2000 9495 10.0U CSENT

FIGURE 6

70	Rpg1 (T)	Reaction to *Puccinia graminis* 1
67	Run1	Reaction to *Ustilago nuda* 1
61	brh1 (br, ari-i)	Brachytic 1 (Breviaristatum-i)
52	fch12 (f_c)	Chlorina seedling 12 (Chlorina seedling c)
50	wax (wx)	Waxy endosperm
48	gsh3 (cer-a)	Glossy sheath 3 (Eceriferum-a) - - ++
43	fch5 (f5)	Chlorina seedling 5
39	yvs2 (y_c)	Virescent seedling 2 (Yellow seedling c)
36	cer-ze (gl5)	Eceriferum-ze (Glossy leaf 5) ++ ++ -
32	wnd	Winding dwarf
	— rsm1 (sm)	Reaction to BSMV 1
26	abo7 (a_{c2})	Albino seedling 7 (Albino seedling c2)
23	ant1 (Rs)	Anthocyanin-less 1 (Red stem)
22	ert-m	Erectoides-m
18	ert-a	Erectoides-a
13	ert-d	Erectoides-d
12	fch8 (f8)	Chlorina seedling 8
10	fst3 (fs3)	Fragile stem 3
9	cer-f	Eceriferum-f + + ++
8	dsp1 (l)	Dense spike 1
7	msg14	Male sterile genetic 14
6	msg10	Male sterile genetic 10
4	sex6	Shrunken endosperm xenia 6
2	seg5	Shrunken endosperm 5
1	seg2	Shrunken endosperm 2
0	nud (n)	Naked caryopsis
-5	fch4 (f4)	Chlorina seedling 4
-6	Amy2	Alpha-amylase 2
-7	lks2 (lk2)	Short awn 2
-8	ubs4 (u4, ari-d)	Unbranched style 4 (Breviaristatum-d)
-11	blx2 (bl2)	Non-blue aleurone xenia 2
-20	lbi3 (lb3)	Long basal rachis internode 3
	— xnt4 (x_{c2})	Xantha seedling 4 (Xantha seedling c2)
	— msg50	Male sterile genetic 50
-31	Rym2 (Ym2)	Reaction to BaYMV 2
-35	seg4	Shrunken endosperm 4
-46	Xnt1 (X_a)	Xantha seedling 1 (Xantha a)
-56	Rph3 (Pa3)	Reaction to *Puccinia hordei* 3
	—xnt9 (xan,,i)	Xantha seedling 9 (Xantha seedling i)
-80	seg1	Shrunken endosperm 1
-85	msg23	Male sterile genetic 23

FIGURE 7

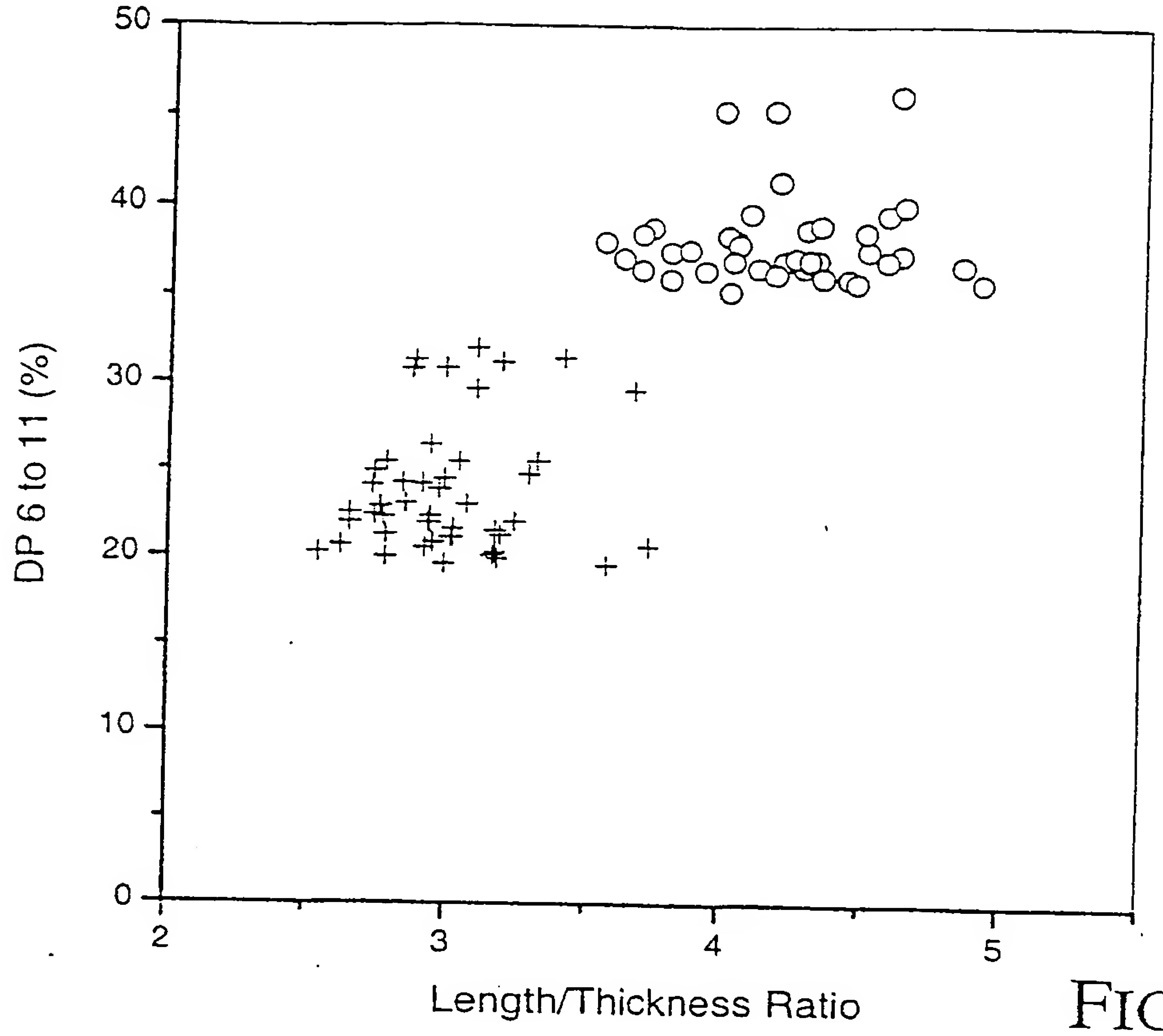
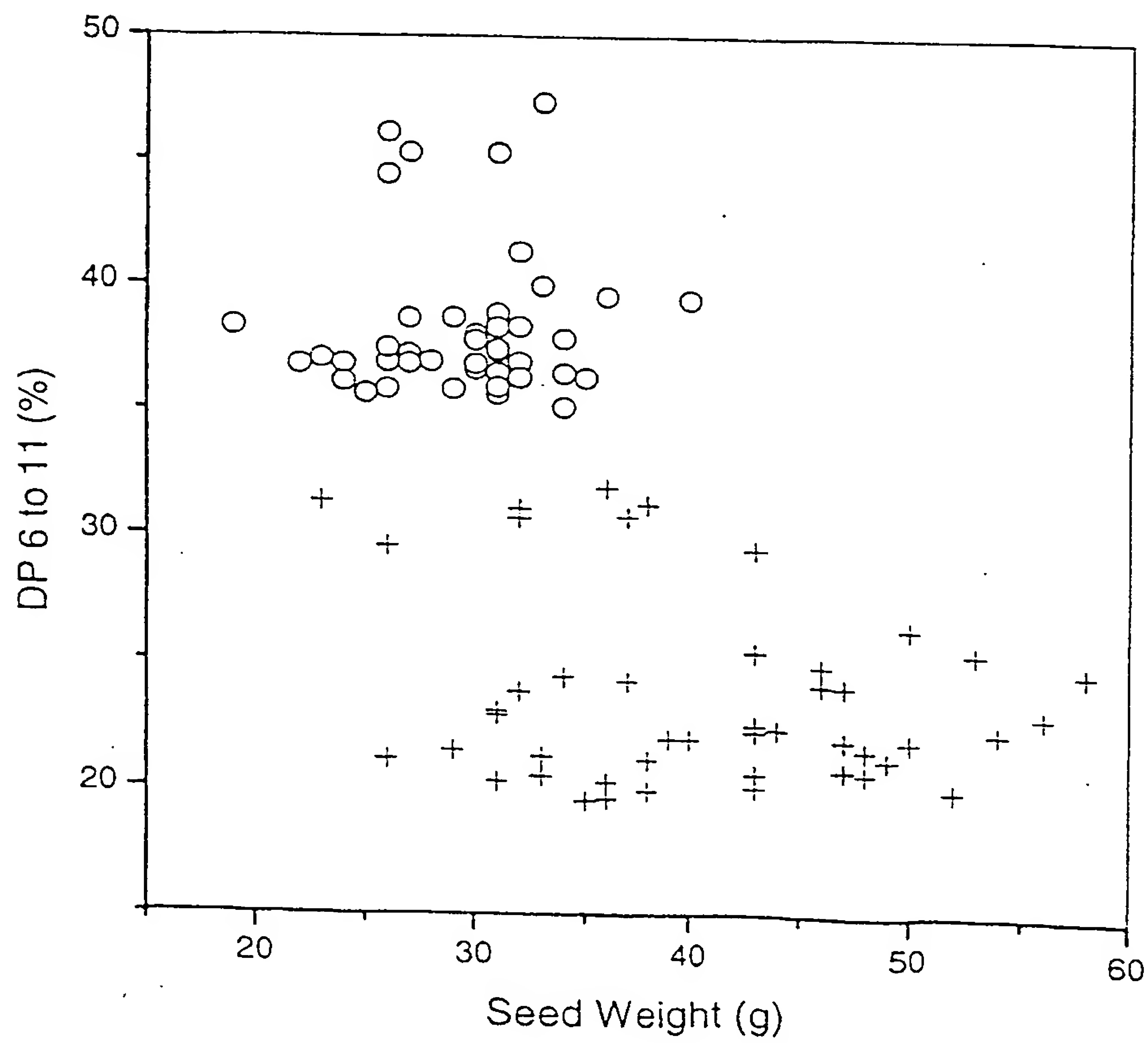


FIGURE 8



Barley SSII cDNA Sequence **FIGURE 9**

```
   1  CCTCGAGGTG CGTTTACCCC ACACAGAGTA CACTCCAACT CCAGTCCAAT
  51  CCAGCCCACT GCCGCTTCTG CCCGCCCATC GTACCGTCGC CCGCCCCGAT
 101  CCCGGCCGCC GCCATGTCGT CGGCGGTCGC GTCCCCCGCG TCCTTCCTCG
 151  CGCTCGCGTC CGCCTCGCCC GGGAGATCAT CACGGAGGAG GGCGAGGGTG
 201  GGCGCGTCGC CAACCCGCGC TGGGGCCGGC AGGCTGCAAT GGCGGCCGTC
 251  GCCGCTGCAG CGCACGGCTC GCGACGGAGC GGTGGCCGCG CGCGCCGCCG
 301  GGATCGACGA CGCCGCGCCC GGTAGGCAGC CCCGCGCTCG CCGCTATGGC
 351  GCCGCCACCA AGGTCGCGGA TCCCGTCAAG ACGCTCGATC GCGACGCCGC
 401  GGAAGGTGGT GGGCCGTCCC CGCCGGCACC GAGGCAGGAC GCCGCCCGTC
 451  TGCCGAGTAA GAACGGCACG CTGATCAACG GTGAGAACAA ACCTACCGGC
 501  GGCGGTGGCG CGACTAAAGA CAGCGGGCTT GCCACACCCG CACGCGCGCC
 551  CCATCTGTCA ATCCAAAACA GAGTACCGGT GAACGGTGAA AACAAACATA
 601  AGGTCGCCTC GCCGCCGACC AGCATAGTGG ATGTCGCGTC TCCGGGTTCC
 651  GCAGCTAACA TTTCCATCAG TAACAAGGTG CCGCCGTCCG TTGTCCCAGC
 701  CAAGAAGACG CCGCCGTCGT CCGTTTTCCC GGCCAAGAAG ACGCTGCCGT
 751  CGTCCGGCTC AAATTTTGTG TCCTCGGCCT CTGCTCCCAG GCTGGACACT
 801  GTCAGCGATG TGGAACTTGC ACAGAAGAAG GATGCGCTGA TTGTCAAAGA
 851  AGCTCCAAAA CCAAAGGCTC TTTCGGCCCC TGCAGCCCCC GCTGTACAAG
 901  AAGACCTTTG GGATTTCAAG AAATACATTG GTTTCGAGGA GCCCGTGGAG
 951  GCCAAGGATG ATGGCTCGGC TGTTGCAGAT GATGCGGGTT CCTTTGAACA
1001  TCACCAGAAT CATGATTCCG GACCTTTGGC AGGGGAGAAC GTCATGAACG
1051  TGGTCGTCGT TGCTGCTGAA TGTTCTCCCT GGTGCAAAAC AGGTGGTCTT
1101  GGAGATGTTG CGGGTGCTTT GCCCAAGGCT TTGGCTAAGA GAGGACATCG
1151  TGTTATGGTT GTGGTACCAA GGTATGGGGA CTATGAGGAA GCCTACGATG
1201  TCGGAGTCCG AAAATACTAC AAGGCTGCTG GACAGGATAT GGAAGTGAAT
1251  TATTTCCATG CTTATATCGA TGGAGTGGAT TTTGTGTTCA TTGACGCTCC
1301  TCTCTTCCGA CACCGTCAGC AAGACATTTA TGGGGGCAGC AGACAGGAAA
1351  TTATGAAGCG CATGATTTTG TTCTGCAAGG CCGCTGTCGA GGTTCCTTGG
1401  CACGTTCCAT GCGGCGGTGT CCCTTACGGG GATGGAAATC TGGTCTTCAT
1451  TGCAAATGAT TGGCACACGG CACTCCTGCC TGTCTATCTG AAAGCATATT
1501  ACAGGGACCA TGGTTTGATG CAATACAGTC GCTCCGTTAT GGTGATACAT
1551  AACATCGCTC ACCAGGGCCG TGGCCCTGTA GATGAATTCC CGTTCACCGA
1601  GTTGCCTGAG CACTACCTGG AACACTTCAG ACTGTACGAC CCCGTCGGCG
1651  GTGAGCACGC CAACTACTTC GCCGCCGGCC TGAAGATGGC GGACCAGGTT
1701  GTCGTCGTGA GCCCCGGGTA CCTGTGGGAG CTGAAGACGG TGGAGGGCGG
1751  CTGGGGGCTT CACGACATCA TACGGCAGAA CGACTGGAAG ACCCGCGGCA
1801  TCGTGAACGG CATCGACAAC ATGGAGTGGA ACCCTGAGGT GGACGTCCAC
1851  CTGAAGTCGG ACGGCTACAC CAACTTCTCC CTGAAGACGC TGGACTCCGG
1901  CAAGCGGCAG TGCAAGGAGG CCCTGCAGCG CGAGCTGGGG CTGCAGGTCC
1951  GCGGCGACGT GCCGCTGCTC GGGTTCATCG GGCGGCTGGA CGGGCAGAAG
2001  GGCGTGGAGA TCATCGCGGA CGCGATGCCC TGGATCGTGA GCCAGGACGT
2051  GCAGCTGGTG ATGCTGGGCA CGGGGCGCCA CGACCTGGAG AGCATGCTGC
2101  AGCACTTCGA GCGGGAGCAC CACGACAAGG TGCGCGGGTG GGTGGGGTTC
2151  TCCGTGCGCC TGGCGCACCG GATCACGGCG GGCGCCGACG CGCTCCTCAT
2201  GCCCTCCCGG TTCGAGCCGT GCGGGCTGAA CCAGCTCTAC GCGATGGCCT
2251  ACGGCACCGT CCCCGTCGTG CACGCCGTCG GCGGCTTGAG GGATACCGTG
2301  CCGCCGTTCG ACCCCTTCAA CCACTCCGGG CTCGGGTGGA CGTTCGACCG
2351  CGCCGAGGCG CACAAGCTGA TCGAGGCGCT CGGGCACTGC CTCCGCACCT
2401  ACCGGGACCA CAAGGAGAGC TGGAGGGGCC TCCAGGAGCG CGGCATGTCG
2451  CAGGACTTCA GCTGGGAACA TGCCGCCAAG CTCTACGAGG ACGTCCTCGT
2501  CCAGGCCAAG TACCAGTGGT GAACGCTGCT ACCCGGTCCA GCCCCGCATG
2551  CGTGCATGAG AGGATGGAAA TGCGCATTGC GCACTTGCAG ATTTGGCGCA
2601  CGCAGGAACG TGCCGTCCTT CTTGATGAGA ACGCCGGCAT CCGCGAGGTT
2651  GAGACGCTGA TTCCGATCTG GTCCGTCGCA GAGTAGAGTG AAACGCTCCT
2701  TGTTGCAGGT ATATGGGAAT GTTTTTTTTC CTTTTTTTTT GCGAGGGAGG
2751  TATATGGGAA TGTTAACTTG GTATTGTAAT GTGGTATGCT GTGTGCATTA
2801  TTACATCGGT TGTTGTTGCT TATTCTTGCT AGCTAAGTCG GAGGCCAAGA
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2851 GCGAAAGCTA GCTCACATGT CTGATGTATG CAAGTGACAT GGTTGGTTTG
2901 AAAAAAAAAA AAAAAAAAAA

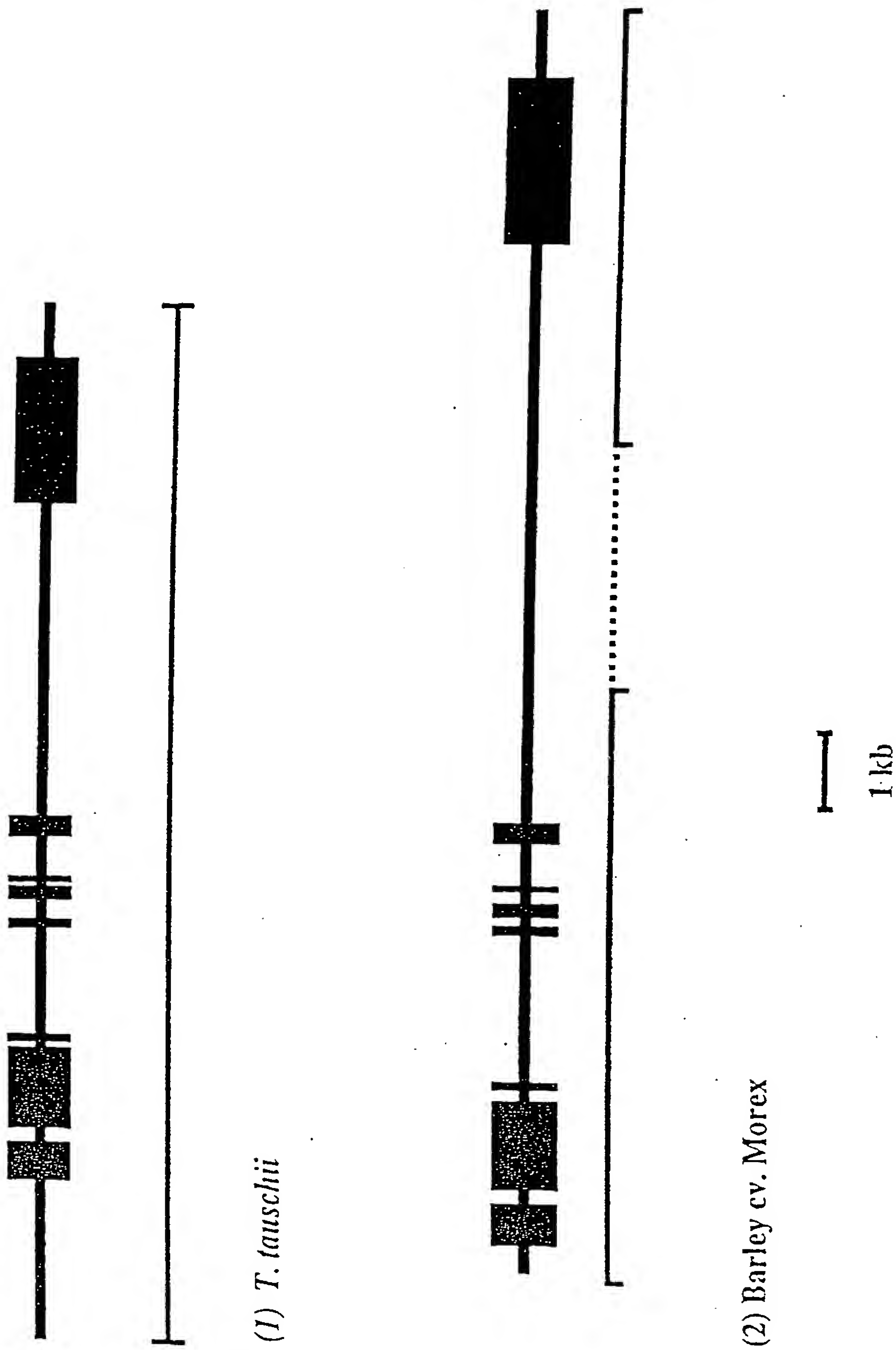

FIGURE 10

Comparison of cDNA Sequences

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          1                                                 50
MK6827    -------GTG CGTTTACCCC ACACAGAGTA CACTCCAACT CCAGTCCAGT
MOREX     -------GTG CGTTTACCCC ACACAGAGTA CACTCCAACT CCAGTCCAGT
292       -------GTG CGTTTACCCC ACACAGAGTA CACTCCAACT CCAGTCCAGT
HIMALAYA  CCTCGAGGTG CGTTTACCCC ACACAGAGTA CACTCCAACT CCAGTCCAAT

          51                                               100
MK6827    CCAGCCCACT GCCGCTTCTG CCCGCCCATC GTACCGTCGC CCGCCCCGAT
MOREX     CCAGCCCACT GCCGCTTCTG CCCGCCCATC GTACCGTCGC CCGCCCCGAT
292       CCAGCCCACT GCCGCTTCTG CCCGCCCATC GTACCGTCGC CCGCCCCGAT
HIMALAYA  CCAGCCCACT GCCGCTTCTG CCCGCCCATC GTACCGTCGC CCGCCCCGAT

          101            *** start codon                   150
MK6827    CCCGGCCGCC GCCATGTCGT CGGCGGTCGC GTCCCCCGCG TCCTTCCTCG
MOREX     CCCGGCCGCC GCCATGTCGT CGGCGGTCGC GTCCCCCGCG TCCTTCCTCG
292       CCCGGCCGCC GCCATGTCGT CGGCGGTCGC GTCCCCCGCG TCCTTCCTCG
HIMALAYA  CCCGGCCGCC GCCATGTCGT CGGCGGTCGC GTCCCCCGCG TCCTTCCTCG

          151                                              200
MK6827    CGCTCGCGTC CGCCTCGCCC GGGAGATCAT CACGGAGGAG GGCGAGGGTG
MOREX     CGCTCGCGTC CGCCTCGCCC GGGAGATCAT CACGGAGGAG GGCGAGGGTG
292       CGCTCGCGTC CGCCTCGCCC GGGAGATCAT CACGGAGGAG GGCGAGGGTG
HIMALAYA  CGCTCGCGTC CGCCTCGCCC GGGAGATCAT CACGGAGGAG GGCGAGGGTG

          201                                     #
MK6827    GGCGCGTCGC CAACCCGCGC TGGGGCCGGC AGGCTGCAAT GACGGCCGTC
MOREX     GGCGCGTCGC CAACCCGCGC TGGGGCCGGC AGGCTGCAAT GGCGGCCGTC
292       GGCGCGTCGC CAACCCGCGC TGGGGCCGGC AGGCTGCAAT GGCGGCCGTC
HIMALAYA  GGCGCGTCGC CAACCCGCGC TGGGGCCGGC AGGCTGCAAT GGCGGCCGTC

          251                                              300
MK6827    GCCGCTGCAG CGCACGGCTC GCGACGGAGC GGTGGCCGCG CGCGCCGCCG
MOREX     GCCGCTGCAG CGCACGGCTC GCGACGGAGC GGTGGCCGCG CGCGCCGCCG
292       GCCGCTGCAG CGCACGGCTC GCGACGGAGC GGTGGCCGCG CGCGCCGCCG
HIMALAYA  GCCGCTGCAG CGCACGGCTC GCGACGGAGC GGTGGCCGCG CGCGCCGCCG

          301                                              350
MK6827    GGATCGACGA CGCCGCGCCC GGTAGGCAGC CCCGCGCTCG CCGCTATGGC
MOREX     GGATCGACGA CGCCGCGCCC GGTAGGCAGC CCCGCGCTCG CCGCTATGGC
292       GGATCGACGA CGCCGCGCCC GGTAGGCAGC CCCGCGCTCG CCGCTATGGC
HIMALAYA  GGATCGACGA CGCCGCGCCC GGTAGGCAGC CCCGCGCTCG CCGCTATGGC

          351                                              400
MK6827    GCCGCCACCA AGGTCGCGGA TCCCGTCAAG ACGCTCGATC GCGACGCCGC
MOREX     GCCGCCACCA AGGTCGCGGA TCCCGTCAAG ACGCTCGATC GCGACGCCGC
292       GCCGCCACCA AGGTCGCGGA TCCCGTCAAG ACGCTCGATC GCGACGCCGC
HIMALAYA  GCCGCCACCA AGGTCGCGGA TCCCGTCAAG ACGCTCGATC GCGACGCCGC

          401                                              450
MK6827    GGAAGGTGGT GGGCCGTCCC CGCCGGCACC GAGGCAGGAC GCCGCCCGTC
MOREX     GGAAGGTGGT GGGCCGTCCC CGCCGGCACC GAGGCAGGAC GCCGCCCGTC
292       GGAAGGTGGT GGGCCGTCCC CGCCGGCACC GAGGCAGGAC GCCGCCCGTC
HIMALAYA  GGAAGGTGGT GGGCCGTCCC CGCCGGCACC GAGGCAGGAC GCCGCCCGTC

          451                                              500
MK6827    TGCCGAGTAA GAACGGCACG CTGATCAACG GTGAGAACAA ACCTACCGGC
MOREX     TGCCGAGTAA GAACGGCACG CTGATCAACG GTGAGAACAA ACCTACCGGC
292       TGCCGAGTAA GAACGGCACG CTGATCAACG GTGAGAACAA ACCTACCGGC
HIMALAYA  TGCCGAGTAA GAACGGCACG CTGATCAACG GTGAGAACAA ACCTACCGGC
```

FIGURE 11

	501				550
MK6827	GGCGGTGGCG	CGACTAAAGA	CAGCGGGCTG	CCCACACCCG	CACGCGCGCC
MOREX	GGCGGTGGCG	CGACTAAAGA	CAGCGGGCTG	CCCACACCCG	CACGCGCGCC
292	GGCGGTGGCG	CGACTAAAGA	CAGCGGGCTG	CCCACACCCG	CACGCGCGCC
HIMALAYA	GGCGGTGGCG	CGACTAAAGA	CAGCGGGCTT	GCCACACCCG	CACGCGCGCC

	551				600
MK6827	CCATCTGTCA	ATCCAGAACA	GAGTACCGGT	GAACGGTGAA	AACAAACATA
MOREX	CCATCTGTCA	ATCCAGAACA	GAGTACCGGT	GAACGGTGAA	AACAAACATA
292	CCATCTGTCA	ATCCAGAACA	GAGTACCGGT	GAACGGTGAA	AACAAACATA
HIMALAYA	CCATCTGTCA	ATCCAAAACA	GAGTACCGGT	GAACGGTGAA	AACAAACATA

	601				650
MK6827	AGGTCGCCTC	GCCGCCGACC	AGCATAGTGG	ATGTCGCGTC	TCCGGGTTCC
MOREX	AGGTCGCCTC	GCCGCCGACC	AGCATAGTGG	ATGTCGCGTC	TCCGGGTTCC
292	AGGTCGCCTC	GCCGCCGACC	AGCATAGTGG	ATGTCGCGTC	TCCGGGTTCC
HIMALAYA	AGGTCGCCTC	GCCGCCGACC	AGCATAGTGG	ATGTCGCGTC	TCCGGGTTCC

	651				700
MK6827	GCAGCTAACA	TTTCCATCAG	TAACAAGGTG	CCGCCGTCCG	TTGTCCCAGC
MOREX	GCAGCTAACA	TTTCCATCAG	TAACAAGGTG	CCGCCGTCCG	TTGTCCCAGC
292	GCAGCCAACA	TTTCCATCAG	TAACAAGGTG	CCGCCGTCCG	TTGTCCCAGC
HIMALAYA	GCAGCTAACA	TTTCCATCAG	TAACAAGGTG	CCGCCGTCCG	TTGTCCCAGC

	701				750
MK6827	CAAGAAGACG	CCGCCGTCGT	CCGTTTTCCC	GGCCAAGAAG	GCGCCGCCGT
MOREX	CAAGAAGACG	CCGCCGTCGT	CCGTTTTCCC	GGCCAAGAAG	GCGCCGCCGT
292	CAAGAAGACG	CCGCCGTCGT	CCGTTTTCCC	GGCCAAGAAG	GCGCCGCCGT
HIMALAYA	CAAGAAGACG	CCGCCGTCGT	CCGTTTTCCC	GGCCAAGAAG	....

	751				800
MK6827	CGTCCGTTGT	CCCGGCCAAG	AAGACGCTGC	CGTCGTCCGG	CTCAAATTTT
MOREX	CGTCCGTTGT	CCCGGCCAAG	AAGACGCTGC	CGTCGTCCGG	CTCAAATTTT
292	CGTCCGTTGT	CCCGGCCAAG	AAGACGCTGC	CGTCGTCCGG	CTCAAATTTT
HIMALAYA	....	....	...ACGCTGC	CGTCGTCCGG	CTCAAATTTT

	801				850
MK6827	GTGTCCTCGG	CCTCTGCTCC	CAGGCTGGAC	ACTGTCAGCG	ATGTGGAACT
MOREX	GTGTCCTCGG	CCTCTGCTCC	CAGGCTGGAC	ACTGTCAGCG	ATGTGGAACT
292	GTGTCCTCGG	CCTCTGCTCC	CAGGCTGGAC	ACTGTCAGCG	ATGTGGAACT
HIMALAYA	GTGTCCTCGG	CCTCTGCTCC	CAGGCTGGAC	ACTGTCAGCG	ATGTGGAACT

	851				900
MK6827	TGCACAGAAG	AAGGATGCGC	TGATTGTCAA	AGAAGCTCCA	AAACCAAAGG
MOREX	TGCACAGAAG	AAGGATGCGC	TGATTGTCAA	AGAAGCTCCA	AAACCAAAGG
292	TGCACAGAAG	AAGGATGCGC	TGATTGTCAA	AGAAGCTCCA	AAACCAAAGG
HIMALAYA	TGCACAGAAG	AAGGATGCGC	TGATTGTCAA	AGAAGCTCCA	AAACCAAAGG

	901				950
MK6827	CTCTTTCGGC	CCCTGCAGCC	CCCGCTGTAC	AAGAAGACCT	TTGGGATTTC
MOREX	CTCTTTCGGC	CCCTGCAGCC	CCCGCTGTAC	AAGAAGACCT	TTGGGATTTC
292	CTCTTTCGGC	CCCTGCAGCC	CCCGCTGTAC	AAGAAGACCT	TTGGGATTTC
HIMALAYA	CTCTTTCGGC	CCCTGCAGCC	CCCGCTGTAC	AAGAAGACCT	TTGGGATTTC

	951				1000
MK6827	AAGAAATACA	TTGGTTTCGA	GGAGCCCGTG	GAGGCCAAGG	ATGATGGCTC
MOREX	AAGAAATACA	TTGGTTTCGA	GGAGCCCGTG	GAGGCCAAGG	ATGATGGCTC
292	AAGAAATACA	TTGGTTTCGA	GGAGCCCGTG	GAGGCCAAGG	ATGATGGCTC
HIMALAYA	AAGAAATACA	TTGGTTTCGA	GGAGCCCGTG	GAGGCCAAGG	ATGATGGCTC

```
          1001                                                1050
MK6827    GGCTGTTGCA GATGATGCGG GTTCCTTTGA ACATCACCAG AATCATGATT
MOREX     GGCTGTTGCA GATGATGCGG GTTCCTTTGA ACATCACCAG AATCATGATT
292       GGCTGTTGCA GATGATGCGG GTTCCTTTGA ACATCACCAG AATCATGATT
HIMALAYA  GGCTGTTGCA GATGATGCGG GTTCCTTTGA ACATCACCAG AATCATGATT

          1051                                                1100
MK6827    CCGGACCTTT GGCAGGGGAG AACGTCATGA ACGTGGTCGT CGTTGCTGCT
MOREX     CCGGACCTTT GGCAGGGGAG AACGTCATGA ACGTGGTCGT CGTTGCTGCT
292       CCGGACCTTT GGCAGGGGAG AACGTCATGA ACGTGGTCGT CGTTGCTGCT
HIMALAYA  CCGGACCTTT GGCAGGGGAG AACGTCATGA ACGTGGTCGT CGTTGCTGCT

          1101                                                1150
MK6827    GAATGTTCTC CCTGGTGCAA AACAGGTGGT CTTGGAGATA TTGCGGGTGC
MOREX     GAATGTTCTC CCTGGTGCAA AACAGGTGGT CTTGGAGATG TTGCGGGTGC
292       GAATGTTCTC CCTGGTGCAA AACAGGTGGT CTTGGAGATG TTGCGGGTGC
HIMALAYA  GAATGTTCTC CCTGGTGCAA AACAGGTGGT CTTGGAGATG TTGCGGGTGC

          1151                                                1200
MK6827    TTTGCCCAAG GCTTTGGCTA AGAGAGGACA TCGTGTTATG GTTGTGGTAC
MOREX     TTTGCCCAAG GCTTTGGCTA AGAGAGGACA TCGTGTTATG GTTGTGGTAC
292       TTTGCCCAAG GCTTTGGCTA AGAGAGGACA TCGTGTTATG GTTGTGGTAC
HIMALAYA  TTTGCCCAAG GCTTTGGCTA AGAGAGGACA TCGTGTTATG GTTGTGGTAC

          1201                                                1250
MK6827    CAAGGTATGG GGACTATGAG GAAGCCTACG ATGTCGGAGT CCGAAAATAC
MOREX     CAAGGTATGG GGACTATGAG GAAGCCTACG ATGTCGGAGT CCGAAAATAC
292       CAAGGTATGG GGACTATGAG GAAGCCTACG ATGTCGGAGT CCGAAAATAC
HIMALAYA  CAAGGTATGG GGACTATGAG GAAGCCTACG ATGTCGGAGT CCGAAAATAC

          1251                                                1300
MK6827    TACAAGGCTG CTGGACAGGA TATGGAAGTG AATTATTTCC ATGCTTATAT
MOREX     TACAAGGCTG CTGGACAGGA TATGGAAGTG AATTATTTCC ATGCTTATAT
292       TACAAGGCTG CTGGACAGGA TATGGAAGTG AATTATTTCC ATGCTTATAT
HIMALAYA  TACAAGGCTG CTGGACAGGA TATGGAAGTG AATTATTTCC ATGCTTATAT

          1301                                                1350
MK6827    CGATGGAGTG GATTTTGTGT TCATTGACGC TCCTCTCTTC CGACACCGTC
MOREX     CGATGGAGTG GATTTTGTGT TCATTGACGC TCCTCTCTTC CGACACCGTC
292       CGATGGAGTG GATTTTGTGT TCATTGACGC TCCTCTCTTC CGACACCGTC
HIMALAYA  CGATGGAGTG GATTTTGTGT TCATTGACGC TCCTCTCTTC CGACACCGTC

          1351                                                1400
MK6827    AGCAAGACAT TTATGGGGGC AGCAGACAGG AAATTATGAA GCGCATGATT
MOREX     AGCAAGACAT TTATGGGGGC AGCAGACAGG AAATTATGAA GCGCATGATT
292       AGCAAGACAT TTATGGGGGC AGCAGACAGG AAATTATGAA GCGCATGATT
HIMALAYA  AGCAAGACAT TTATGGGGGC AGCAGACAGG AAATTATGAA GCGCATGATT

          1401                                                1450
MK6827    TTGTTCTGCA AGGCCGCTGT CGAGGTTCCT TGGCACGTTC CATGCGGCGG
MOREX     TTGTTCTGCA AGGCCGCTGT CGAGGTTCCT TGGCACGTTC CATGCGGCGG
292       TTGTTCTGCA AGGCCGCTGT CGAGGTTCCT TGGCACGTTC CATGCGGCGG
HIMALAYA  TTGTTCTGCA AGGCCGCTGT CGAGGTTCCT TGGCACGTTC CATGCGGCGG

          1451                                                1500
MK6827    TGTCCCTTAC GGGGATGGAA ATCTGGTCTT CATTGCAAAT GATTGGCACA
MOREX     TGTCCCTTAC GGGGATGGAA ATCTGGTCTT CATTGCAAAT GATTGGCACA
292       TGTCCCTTAC GGGGATGGAA ATCTGGTCTT CATTGCAAAT GATTGGCACA
HIMALAYA  TGTCCCTTAC GGGGATGGAA ATCTGGTCTT CATTGCAAAT GATTGGCACA
```

```
           1501                                                   1550
MK6827     CGGCACTCCT GCCTGTCTAT CTGAAAGCAT ATTACAGGGA CCATGGTTTG
MOREX      CGGCACTCCT GCCTGTCTAT CTGAAAGCAT ATTACAGGGA CCATGGTTTG
292        CGGCACTCCT GCCTGTCTAT CTGAAAGCAT ATTACAGGGA CCATGGTTTG
HIMALAYA   CGGCACTCCT GCCTGTCTAT CTGAAAGCAT ATTACAGGGA CCATGGTTTG

           1551                                                   1600
MK6827     ATGCAATACA GTCGCTCCGT TATGGTGATA CATAACATCG CTCACCAGGG
MOREX      ATGCAATACA GTCGCTCCGT TATGGTGATA CATAACATCG CTCACCAGGG
292        ATGCAATACA GTCGCTCCGT TATGGTGATA CATAACATCG CTCACCAGGG
HIMALAYA   ATGCAATACA GTCGCTCCGT TATGGTGATA CATAACATCG CTCACCAGGG

           1601                                                   1650
MK6827     CCGTGGCCCT GTAGATGAAT TCCCGTTCAC CGAGTTGCCT GAGCACTACC
MOREX      CCGTGGCCCT GTAGATGAAT TCCCGTTCAC CGAGTTGCCT GAGCACTACC
292        CCGTGGCCCT GTAGATGAAT TCCCGTTCAC CGAGTTGCCT GAGCACTACC
HIMALAYA   CCGTGGCCCT GTAGATGAAT TCCCGTTCAC CGAGTTGCCT GAGCACTACC

           1651                                                   1700
MK6827     TGGAACACTT CAGACTGTAC GACCCCGTCG GCGGTGAGCA CGCCAACTAC
MOREX      TGGAACACTT CAGACTGTAC GACCCCGTCG GCGGTGAGCA CGCCAACTAC
292        TGGAACACTT CAGACTGTAC GACCCCGTCG GCGGTGAGCA CGCCAACTAC
HIMALAYA   TGGAACACTT CAGACTGTAC GACCCCGTCG GCGGTGAGCA CGCCAACTAC

           1701                                                   1750
MK6827     TTCGCCGCCG GCCTGAAGAT GGCGGACCAG GTTGTCGTCG TGAGCCCCGG
MOREX      TTCGCCGCCG GCCTGAAGAT GGCGGACCAG GTTGTCGTCG TGAGCCCCGG
292        TTCGCCGCCG GCCTGAAGAT GGCGGACCAG GTTGTCGTCG TGAGCCCCGG
HIMALAYA   TTCGCCGCCG GCCTGAAGAT GGCGGACCAG GTTGTCGTCG TGAGCCCCGG

           1751                                                   1800
MK6827     GTACCTGTGG GAGCTGAAGA CGGTGGAGGG CGGCTGGGGG CTTCACGACA
MOREX      GTACCTGTGG GAGCTGAAGA CGGTGGAGGG CGGCTGGGGG CTTCACGACA
292        GTACCTGTGG GAGCTGAAGA CGGTGGAGGG CGGCTGGGGG CTTCACGACA
HIMALAYA   GTACCTGTGG GAGCTGAAGA CGGTGGAGGG CGGCTGGGGG CTTCACGACA

           1801                                                   1850
MK6827     TCATACGGCA GAACGACTGG AAGACCCGCG GCATCGTGAA CGGCATCGAC
MOREX      TCATACGGCA GAACGACTGG AAGACCCGCG GCATCGTGAA CGGCATCGAC
292        TCATACGGCA GAACGACTGG AAGACCCGCG GCATCGTGAA CGGCATCGAC
HIMALAYA   TCATACGGCA GAACGACTGG AAGACCCGCG GCATCGTGAA CGGCATCGAC

           1851           &                           1900
MK6827     AACATGGAGT GGAACCCTGA GGTGGACGTC CACCTGAAGT CGGACGGCTA
MOREX      AACATGGAGT GGAACCCTGA GGTGGACGTC CACCTGAAGT CGGACGGCTA
292        AACATGGAGT GAAACCCTGA GGTGGACGTC CACCTGAAGT CGGACGGCTA
HIMALAYA   AACATGGAGT GGAACCCTGA GGTGGACGTC CACCTGAAGT CGGACGGCTA

           1901                                                   1950
MK6827     CACCAACTTC TCCCTGAAGA CGCTGGACTC CGGCAAGCGG CAGTGCAAGG
MOREX      CACCAACTTC TCCCTGAAGA CGCTGGACTC CGGCAAGCGG CAGTGCAAGG
292        CACCAACTTC TCCCTGAAGA CGCTGGACTC CGGCAAGCGG CAGTGCAAGG
HIMALAYA   CACCAACTTC TCCCTGAAGA CGCTGGACTC CGGCAAGCGG CAGTGCAAGG

           1951                                                   2000
MK6827     AGGCCCTGCA GCGCGAGCTG GGGCTGCAGG TCCGCGGCGA CGTGCCGCTG
MOREX      AGGCCCTGCA GCGCGAGCTG GGGCTGCAGG TCCGCGGCGA CGTGCCGCTG
292        AGGCCCTGCA GCGCGAGCTG GGGCTGCAGG TCCGCGGCGA CGTGCCGCTG
HIMALAYA   AGGCCCTGCA GCGCGAGCTG GGGCTGCAGG TCCGCGGCGA CGTGCCGCTG
```

	2001				2050
MK6827	CTCGGGTTCA	TCGGGCGGCT	GGACGGGCAG	AAGGGCGTGG	AGATCATCGC
MOREX	CTCGGGTTCA	TCGGGCGGCT	GGACGGGCAG	AAGGGCGTGG	AGATCATCGC
292	CTCGGGTTCA	TCGGGCGGCT	GGACGGGCAG	AAGGGCGTGG	AGATCATCGC
HIMALAYA	CTCGGGTTCA	TCGGGCGGCT	GGACGGGCAG	AAGGGCGTGG	AGATCATCGC
	2051				2100
MK6827	GGACGCGATG	CCCTGGATCG	TGAGCCAGGA	CGTGCAGCTG	GTGATGCTGG
MOREX	GGACGCGATG	CCCTGGATCG	TGAGCCAGGA	CGTGCAGCTG	GTGATGCTGG
292	GGACGCGATG	CCCTGGATCG	TGAGCCAGGA	CGTGCAGCTG	GTGATGCTGG
HIMALAYA	GGACGCGATG	CCCTGGATCG	TGAGCCAGGA	CGTGCAGCTG	GTGATGCTGG
	2101				2150
MK6827	GCACGGGGCG	CCACGACCTG	GAGAGCATGC	TGCAGCACTT	CGAGCGGGAG
MOREX	GCACGGGGCG	CCACGACCTG	GAGAGCATGC	TGCAGCACTT	CGAGCGGGAG
292	GCACGGGGCG	CCACGACCTG	GAGAGCATGC	TGCAGCACTT	CGAGCGGGAG
HIMALAYA	GCACGGGGCG	CCACGACCTG	GAGAGCATGC	TGCAGCACTT	CGAGCGGGAG
	2151				2200
MK6827	CACCACGACA	AGGTGCGCGG	GTGGGTGGGG	TTCTCCGTGC	GCCTGGCGCA
MOREX	CACCACGACA	AGGTGCGCGG	GTGGGTGGGG	TTCTCCGTGC	GCCTGGCGCA
292	CACCACGACA	AGGTGCGCGG	GTGGGTGGGG	TTCTCCGTGC	GCCTGGCGCA
HIMALAYA	CACCACGACA	AGGTGCGCGG	GTGGGTGGGG	TTCTCCGTGC	GCCTGGCGCA
	2201				2250
MK6827	CCGGATCACG	GCGGGCGCCG	ACGCGCTCCT	CATGCCCTCC	CGGTTCGAGC
MOREX	CCGGATCACG	GCGGGCGCCG	ACGCGCTCCT	CATGCCCTCC	CGGTTCGAGC
292	CCGGATCACG	GCGGGCGCCG	ACGCGCTCCT	CATGCCCTCC	CGGTTCGAGC
HIMALAYA	CCGGATCACG	GCGGGCGCCG	ACGCGCTCCT	CATGCCCTCC	CGGTTCGAGC
	2251				2300
MK6827	CGTGCGGGCT	GAACCAGCTC	TACGCGATGG	CCTACGGCAC	CATCCCTGTC
MOREX	CGTGCGGGCT	GAACCAGCTC	TACGCGATGG	CCTACGGCAC	CATCCCTGTC
292	CGTGCGGGCT	GAACCAGCTC	TACGCGATGG	CCTACGGCAC	CATCCCTGTC
HIMALAYA	CGTGCGGGCT	GAACCAGCTC	TACGCGATGG	CCTACGGCAC	CGTCCCCGTC
	2301				2350
MK6827	GTGCACGCCG	TCGGCGGCCT	GAGGGATACC	GTGCCGCCGT	TCGACCCCTT
MOREX	GTGCACGCCG	TCGGCGGCCT	GAGGGATACC	GTGCCGCCGT	TCGACCCCTT
292	GTGCACGCCG	TCGGCGGCCT	GAGGGATACC	GTGCCGCCGT	TCGACCCCTT
HIMALAYA	GTGCACGCCG	TCGGCGGCTT	GAGGGATACC	GTGCCGCCGT	TCGACCCCTT
	2351				2400
MK6827	CAACCACTCC	GGGCTCGGGT	GGACGTTCGA	CCGCGCCGAG	GCGCACAAGC
MOREX	CAACCACTCC	GGGCTCGGGT	GGACGTTCGA	CCGCGCCGAG	GCGCACAAGC
292	CAACCACTCC	GGGCTCGGGT	GGACGTTCGA	CCGCGCCGAG	GCGCACAAGC
HIMALAYA	CAACCACTCC	GGGCTCGGGT	GGACGTTCGA	CCGCGCCGAG	GCGCACAAGC
	2401				2450
MK6827	TGATCGAGGC	GCTCGGGCAC	TGCCTCCGCA	CCTACCGGGA	CCACAAGGAG
MOREX	TGATCGAGGC	GCTCGGGCAC	TGCCTCCGCA	CCTACCGGGA	CCACAAGGAG
292	TGATCGAGGC	GCTCGGGCAC	TGCCTCCGCA	CCTACCGGGA	CCACAAGGAG
HIMALAYA	TGATCGAGGC	GCTCGGGCAC	TGCCTCCGCA	CCTACCGGGA	CCACAAGGAG
	2451				2500
MK6827	AGCTGGAGGG	GCCTCCAGGA	GCGCGGCATG	TCGCAGGACT	TCAGCTGGGA
MOREX	AGCTGGAGGG	GCCTCCAGGA	GCGCGGCATG	TCGCAGGACT	TCAGCTGGGA
292	AGCTGGAGGG	GCCTCCAGGA	GCGCGGCATG	TCGCAGGACT	TCAGCTGGGA
HIMALAYA	AGCTGGAGGG	GCCTCCAGGA	GCGCGGCATG	TCGCAGGACT	TCAGCTGGGA

	2501				2550
MK6827	ACATGCCGCC	AAGCTCTACG	AGGACGTCCT	CGTCCAGGCC	AAGTACCAGT
MOREX	ACATGCCGCC	AAGCTCTACG	AGGACGTCCT	CGTCCAGGCC	AAGTACCAGT
292	ACATGCCGCC	AAGCTCTACG	AGGACGTCCT	CGTCCAGGCC	AAGTACCAGT
HIMALAYA	ACATGCCGCC	AAGCTCTACG	AGGACGTCCT	CGTCCAGGCC	AAGTACCAGT
	2551				2600
	*** stop codon				
MK6827	GG**TGA**ACGCT	GCTACCCGGT	CCAGCCCCGC	ATGCGTGCAT	GAGAGGATGG
MOREX	GG**TGA**ACGCT	GCTACCCGGT	CCAGCCCCGC	ATGCGTGCAT	GAGAGGATGG
292	GG**TGA**ACGCT	GCTACCCGGT	CCAGCCCCGC	ATGCGTGCAT	GAGAGGATGG
HIMALAYA	GG**TGA**ACGCT	GCTACCCGGT	CCAGCCCCGC	ATGCGTGCAT	GAGAGGATGG
	2601				2650
MK6827	AAATGCGCAT	TGCGCACTTG	CAGATTTGGC	GCATGCAGGA	ACGTGCCGTC
MOREX	AAATGCGCAT	TGCGCACTTG	CAGATTTGGC	GCATGCAGGA	ACGTGCCGTC
292	AAATGCGCAT	TGCGCACTTG	CAGATTTGGC	GCACGCAGGA	ACGTGCCGTC
HIMALAYA	AAATGCGCAT	TGCGCACTTG	CAGATTTGGC	GCACGCAGGA	ACGTGCCGTC
	2651				2700
MK6827	CTTCTTGATG	GGAACGCCGG	CATCCGCGAG	GTTGAGACGC	TGATTCCGAT
MOREX	CTTCTTGATG	GGAACGCCGG	CATCCGCGAG	GTTGAGACGC	TGATTCCGAT
292	CTTCTTGATG	AGAACGCCGG	CATCCGCGAG	GTTGAGACGC	TGATTCCGAT
HIMALAYA	CTTCTTGATG	AGAACGCCGG	CATCCGCGAG	GTTGAGACGC	TGATTCCGAT
	2701				2750
MK6827	CTGGTCCGTC	GCAGAGTAGA	GTGAAACGCT	CCTTGTTGCA	GGTATATGGG
MOREX	CTGGTCCGTC	GCAGAGTAGA	GTGAAACGCT	CCTTGTTGCA	GGTATATGGG
292	CTGGTCCGTC	GCAGAGTAGA	GTGAAACGCT	CCTTGTTGCA	GGTATATGGG
HIMALAYA	CTGGTCCGTC	GCAGAGTAGA	GTGAAACGCT	CCTTGTTGCA	GGTATATGGG
	2751				2800
MK6827	AATGTTTTTT	TTTTCC.TTT	TTTTTTTTGC	GAGGGAGGTA	TATGGGAATG
MOREX	AATGTTTTTT	TTTTCCTTTT	TTTTTTTTGC	GAGGGAGGTA	TATGGGAATG
292	AATGTTTTTT	TT..CC...T	TTTTTTTTGC	GAGGGAGGTA	TATGGGAATG
HIMALAYA	AATGTTTTTT	TT..CC...T	TTTTTTTTGC	GAGGGAGGTA	TATGGGAATG
	2801				2850
MK6827	TTAACTTGGT	ATTGTAATGT	GGTATGCTGT	GTGCATTATT	ACATCGGTTG
MOREX	TTAACTTGGT	ATTGTAATGT	GGTATGCTGT	GTGCATTATT	ACATCGGTTG
292	TTAACTTGGT	ATTGTAATGT	GGTATGCTGT	GTGCATTATT	ACATCGGTTG
HIMALAYA	TTAACTTGGT	ATTGTAATGT	GGTATGCTGT	GTGCATTATT	ACATCGGTTG
	2851				2900
MK6827	TTGTTGCTTA	TTCTTGCTAG	CTAAGTCGGA	GGCCAAGAGC	GAAAGCTAGC
MOREX	TTGTTGCTTA	TTCTTGCTAG	CTAAGTCGGA	GGCCAAGAGC	GAAAGCTAGC
292	TTGTTGCTTA	TTCTTGCTAG	CTAAGTCGGA	GGCCAAGAGC	GAAAGCTAGC
HIMALAYA	TTGTTGCTTA	TTCTTGCTAG	CTAAGTCGGA	GGCCAAGAGC	GAAAGCTAGC
	2901				2950
MK6827	TCACATGTCT	GATGTATGCA	AGTGACATGG	TTGGTTTGGT	TGTGCAGTGC
MOREX	TCACATGTCT	GATGTATGCA	AGTGACATGG	TTGGTTTGGT	TGTGCAGTGC
292	TCACATGTCT	GATGTATGCA	AGTGACATGG	TTGGTTTGGT	TGTGCAGTGC
HIMALAYA	TCACATGTCT	GATGTATGCA	AGTGACATGG	TTGGTTTGAA	AAAAAAAAAA
	2951				
MK6827	AAACGGCA				
MOREX	AAACGGCA				
292	AAACGGCA				
HIMALAYA	AAAAAAAA				

Comparison of SSII Amino Acid Sequences

```
           1                                               MK6827 mutation #
Morex      MSSAVASPAS FLALASASPG RSSRRRARVG ASPTRAGAGR LQWRPSPLQR
Himalaya   MSSAVASPAS FLALASASPG RSSRRRARVG ASPTRAGAGR LQWRPSPLQR
292        MSSAVASPAS FLALASASPG RSSRRRARVG ASPTRAGAGR LQWRPSPLQR
MK6827     MSSAVASPAS FLALASASPG RSSRRRARVG ASPTRAGAGR LQ*RPSPLQR

           51                                                     100
Morex      TARDGAVAAR AAGIDDAAPG RQPRARRYGA ATKVADPVKT LDRDAAEGGG
Himalaya   TARDGAVAAR AAGIDDAAPG RQPRARRYGA ATKVADPVKT LDRDAAEGGG
292        TARDGAVAAR AAGIDDAAPG RQPRARRYGA ATKVADPVKT LDRDAAEGGG
MK6827     TARDGAVAAR AAGIDDAAPG RQPRARRYGA ATKVADPVKT LDRDAAEGGG

           101                                                    150
Morex      PSPPAPRQDA ARLPSKNGTL INGENKPTGG GGATKDSGLP TPARAPHLSI
Himalaya   PSPPAPRQDA ARLPSKNGTL INGENKPTGG GGATKDSGLP TPARAPHLSI
292        PSPPAPRQDA ARLPSKNGTL INGENKPTGG GGATKDSGLP TPARAPHLSI
MK6827     PSPPAPRQDA ARLPSKNGTL INGENKPTGG GGATKDSGLP TPARAPHLSI

           151                                                    200
Morex      QNRVPVNGEN KHKVASPPTS IVDVASPGSA ANISISNKVP PSVVPAKKTP
Himalaya   QNRVPVNGEN KHKVASPPTS IVDVASPGSA ANISISNKVP PSVVPAKKTP
292        QNRVPVNGEN KHKVASPPTS IVDVASPGSA ANISISNKVP PSVVPAKKTP
MK6827     QNRVPVNGEN KHKVASPPTS IVDVASPGSA ANISISNKVP PSVVPAKKTP

           201                                                    250
Morex      PSSVFPAKKT LPSSGSNFVS SASAPRLDTV SDVELAQKKD ALIVKEAPKP
Himalaya   PSSVFPAKKT LPSSGSNFVS SASAPRLDTV SDVELAQKKD ALIVKEAPKP
292        PSSVFPAKKT LPSSGSNFVS SASAPRLDTV SDVELAQKKD ALIVKEAPKP
MK6827     PSSVFPAKKT LPSSGSNFVS SASAPRLDTV SDVELAQKKD ALIVKEAPKP

           251                                                    300
Morex      KALSAPAAPA VQEDLWDFKK YIGFEEPVEA KDDGSAVADD AGSFEHHQNH
Himalaya   KALSAPAAPA VQEDLWDFKK YIGFEEPVEA KDDGSAVADD AGSFEHHQNH
292        KALSAPAAPA VQEDLWDFKK YIGFEEPVEA KDDGSAVADD AGSFEHHQNH
MK6827     KALSAPAAPA VQEDLWDFKK YIGFEEPVEA KDDGSAVADD AGSFEHHQNH

           301                                                    350
Morex      DSGPLAGENV MNVVVVAAEC SPWCKTGGLG DVAGALPKAL AKRGHRVMVV
Himalaya   DSGPLAGENV MNVVVVAAEC SPWCKTGGLG DVAGALPKAL AKRGHRVMVV
292        DSGPLAGENV MNVVVVAAEC SPWCKTGGLG DVAGALPKAL AKRGHRVMVV
MK6827     DSGPLAGENV MNVVVVAAEC SPWCKTGGLG DIAGALPKAL AKRGHRVMVV

           351                                                    400
Morex      VPRYGDYEEA YDVGVRKYYK AAGQDMEVNY FHAYIDGVDF VFIDAPLFRH
Himalaya   VPRYGDYEEA YDVGVRKYYK AAGQDMEVNY FHAYIDGVDF VFIDAPLFRH
292        VPRYGDYEEA YDVGVRKYYK AAGQDMEVNY FHAYIDGVDF VFIDAPLFRH
MK6827     VPRYGDYEEA YDVGVRKYYK AAGQDMEVNY FHAYIDGVDF VFIDAPLFRH

           401                                                    450
Morex      RQQDIYGGSR QEIMKRMILF CKAAVEVPWH VPCGGVPYGD GNLVFIANDW
Himalaya   RQQDIYGGSR QEIMKRMILF CKAAVEVPWH VPCGGVPYGD GNLVFIANDW
292        RQQDIYGGSR QEIMKRMILF CKAAVEVPWH VPCGGVPYGD GNLVFIANDW
MK6827     RQQDIYGGSR QEIMKRMILF CKAAVEVPWH VPCGGVPYGD GNLVFIANDW

           451                                                    500
Morex      HTALLPVYLK AYYRDHGLMQ YSRSVMVIHN IAHQGRGPVD EFPFTELPEH
Himalaya   HTALLPVYLK AYYRDHGLMQ YSRSVMVIHN IAHQGRGPVD EFPFTELPEH
292        HTALLPVYLK AYYRDHGLMQ YSRSVMVIHN IAHQGRGPVD EFPFTELPEH
MK6827     HTALLPVYLK AYYRDHGLMQ YSRSVMVIHN IAHQGRGPVD EFPFTELPEH

           501                                                    550
Morex      YLEHFRLYDP VGGEHANYFA AGLKMADQVV VVSPGYLWEL KTVEGGWGLH
Himalaya   YLEHFRLYDP VGGEHANYFA AGLKMADQVV VVSPGYLWEL KTVEGGWGLH
292        YLEHFRLYDP VGGEHANYFA AGLKMADQVV VVSPGYLWEL KTVEGGWGLH
MK6827     YLEHFRLYDP VGGEHANYFA AGLKMADQVV VVSPGYLWEL KTVEGGWGLH
```

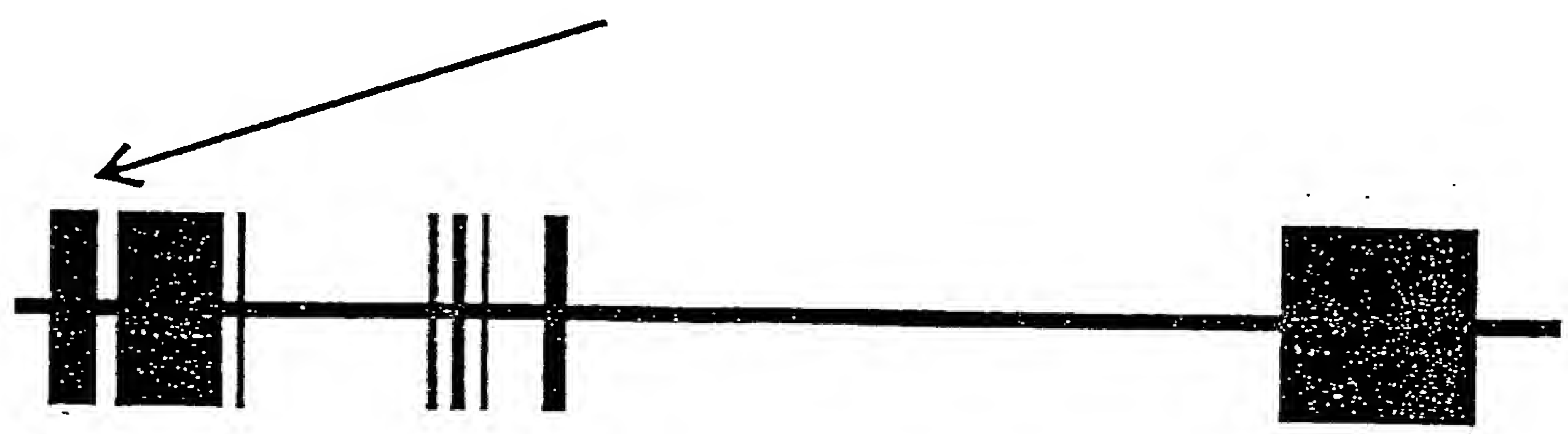
FIGURE 12

```
            551                                  $ 292 mutation                  600
Morex       DIIRQNDWKT RGIVNGIDNM EWNPEVDVHL KSDGYTNFSL KTLDSGKRQC
Himalaya    DIIRQNDWKT RGIVNGIDNM EWNPEVDVHL KSDGYTNFSL KTLDSGKRQC
292         DIIRQNDWKT RGIVNGIDNM E*NPEVDVHL KSDGYTNFSL KTLDSGKRQC
MK6827      DIIRQNDWKT RGIVNGIDNM EWNPEVDVHL KSDGYTNFSL KTLDSGKRQC

            601                                                  650
Morex       KEALQRELGL QVRGDVPLLG FIGRLDGQKG VEIIADAMPW IVSQDVQLVM
Himalaya    KEALQRELGL QVRGDVPLLG FIGRLDGQKG VEIIADAMPW IVSQDVQLVM
292         KEALQRELGL QVRGDVPLLG FIGRLDGQKG VEIIADAMPW IVSQDVQLVM
MK6827      KEALQRELGL QVRGDVPLLG FIGRLDGQKG VEIIADAMPW IVSQDVQLVM

            651                                                  700
Morex       LGTGRHDLES MLQHFEREHH DKVRGWVGFS VRLAHRITAG ADALLMPSRF
Himalaya    LGTGRHDLES MLQHFEREHH DKVRGWVGFS VRLAHRITAG ADALLMPSRF
292         LGTGRHDLES MLQHFEREHH DKVRGWVGFS VRLAHRITAG ADALLMPSRF
MK6827      LGTGRHDLES MLQHFEREHH DKVRGWVGFS VRLAHRITAG ADALLMPSRF

            701                                                  750
Morex       EPCGLNQLYA MAYGTIPVVH AVGGLRDTVP PFDPFNHSGL GWTFDRAEAH
Himalaya    EPCGLNQLYA MAYGTIPVVH AVGGLRDTVP PFDPFNHSGL GWTFDRAEAH
292         EPCGLNQLYA MAYGTIPVVH AVGGLRDTVP PFDPFNHSGL GWTFDRAEAH
MK6827      EPCGLNQLYA MAYGTIPVVH AVGGLRDTVP PFDPFNHSGL GWTFDRAEAH

            751                                                  800
Morex       KLIEALGHCL RTYRDHKESW RGLQERGMSQ DFSWEHAAKL YEDVLVQAKY
Himalaya    KLIEALGHCL RTYRDHKESW RGLQERGMSQ DFSWEHAAKL YEDVLVQAKY
292         KLIEALGHCL RTYRDHKESW RGLQERGMSQ DFSWEHAAKL YEDVLVQAKY
MK6827      KLIEALGHCL RTYRDHKESW RGLQERGMSQ DFSWEHAAKL YEDVLVQAKY

            801
Morex       QW*
Himalaya    QW*
292         QW*
MK6827      QW*
```

Position 242 of barley SSII
cDNA sequence

Himalaya
.....CGGCAGGCTGCAATGGCGGCCGTCGCCGCT....

MK6827
.....CGGCAGGCTGCAATGACGGCCGTCGCCGCT....



Barley SSII
gene structure

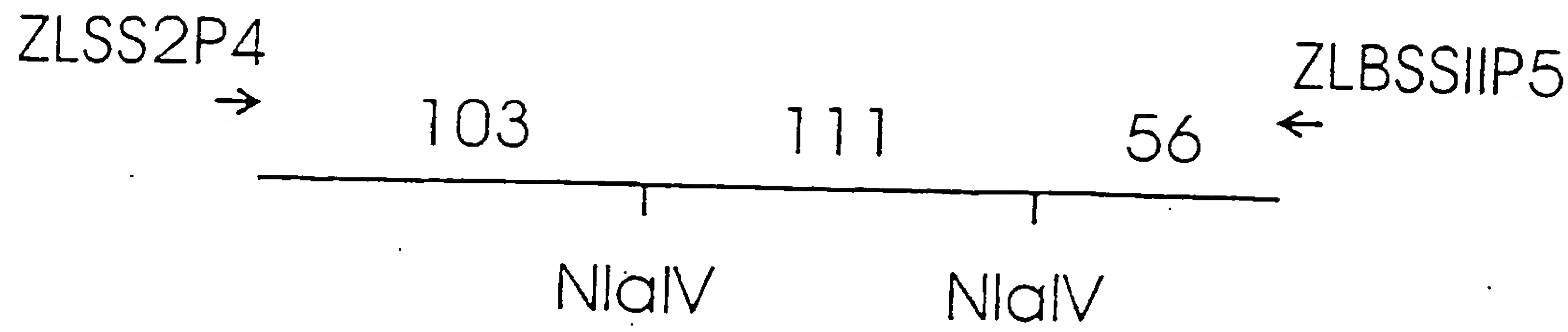
Position 1829 of barley SSII
cDNA sequence

292
.....GACAACATGGAGTGAAACCCTGAGGTGGACGTCCA.....

Himalaya
.....GACAACATGGAGTGGAACCCTGAGGTGGACGTCCA.....

FIGURE 13

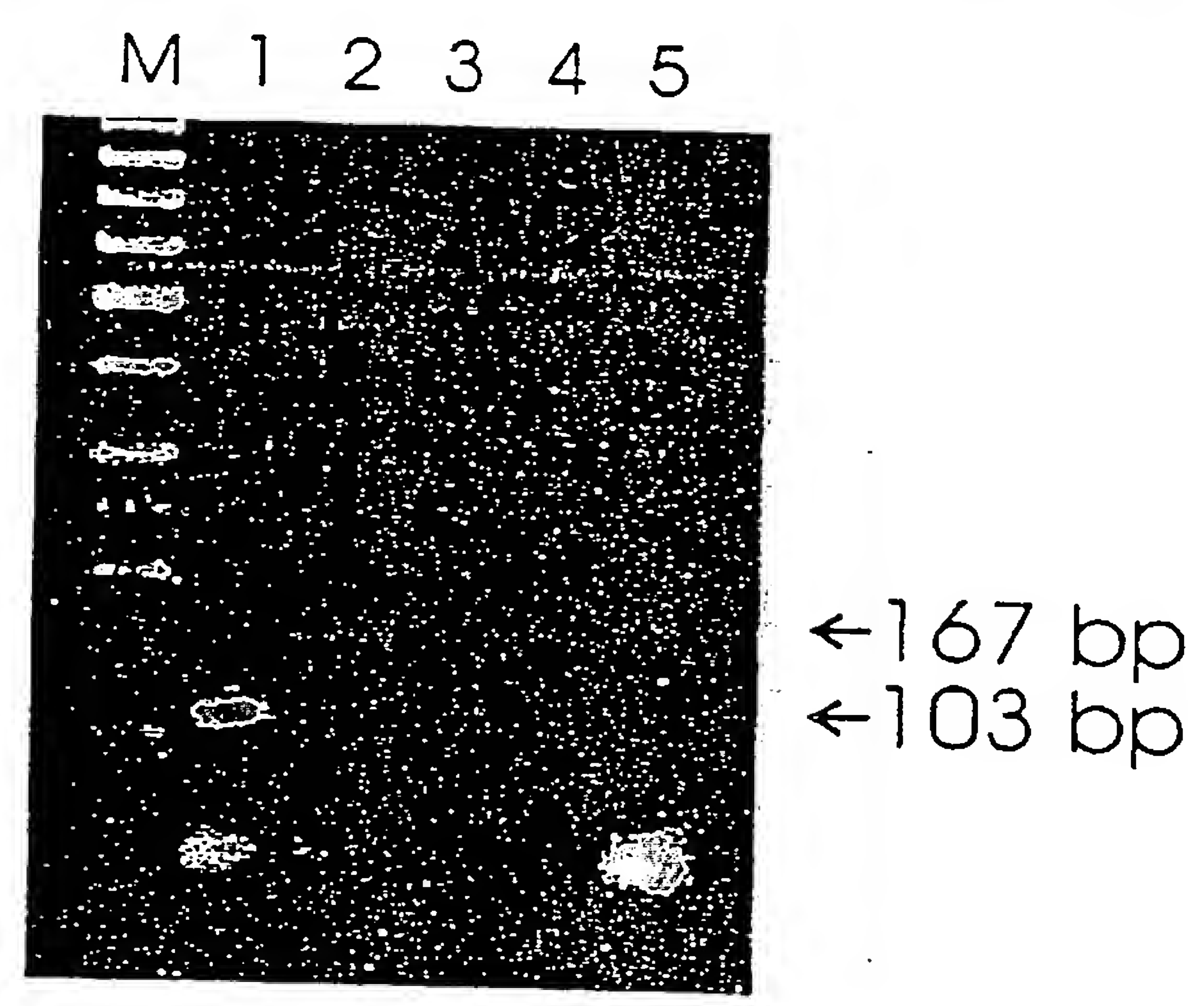
(a)



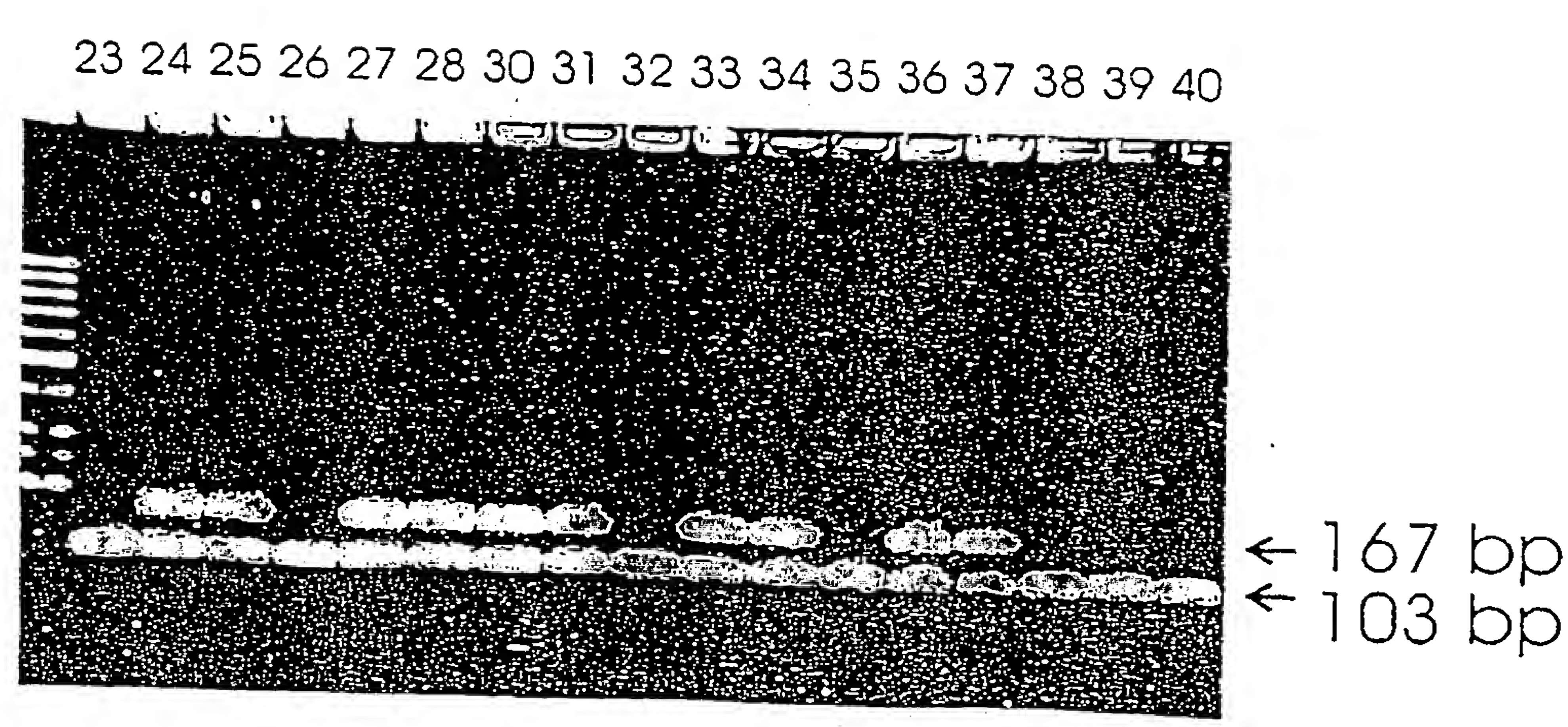
(b)

FIGURE 14

(c)



(d)



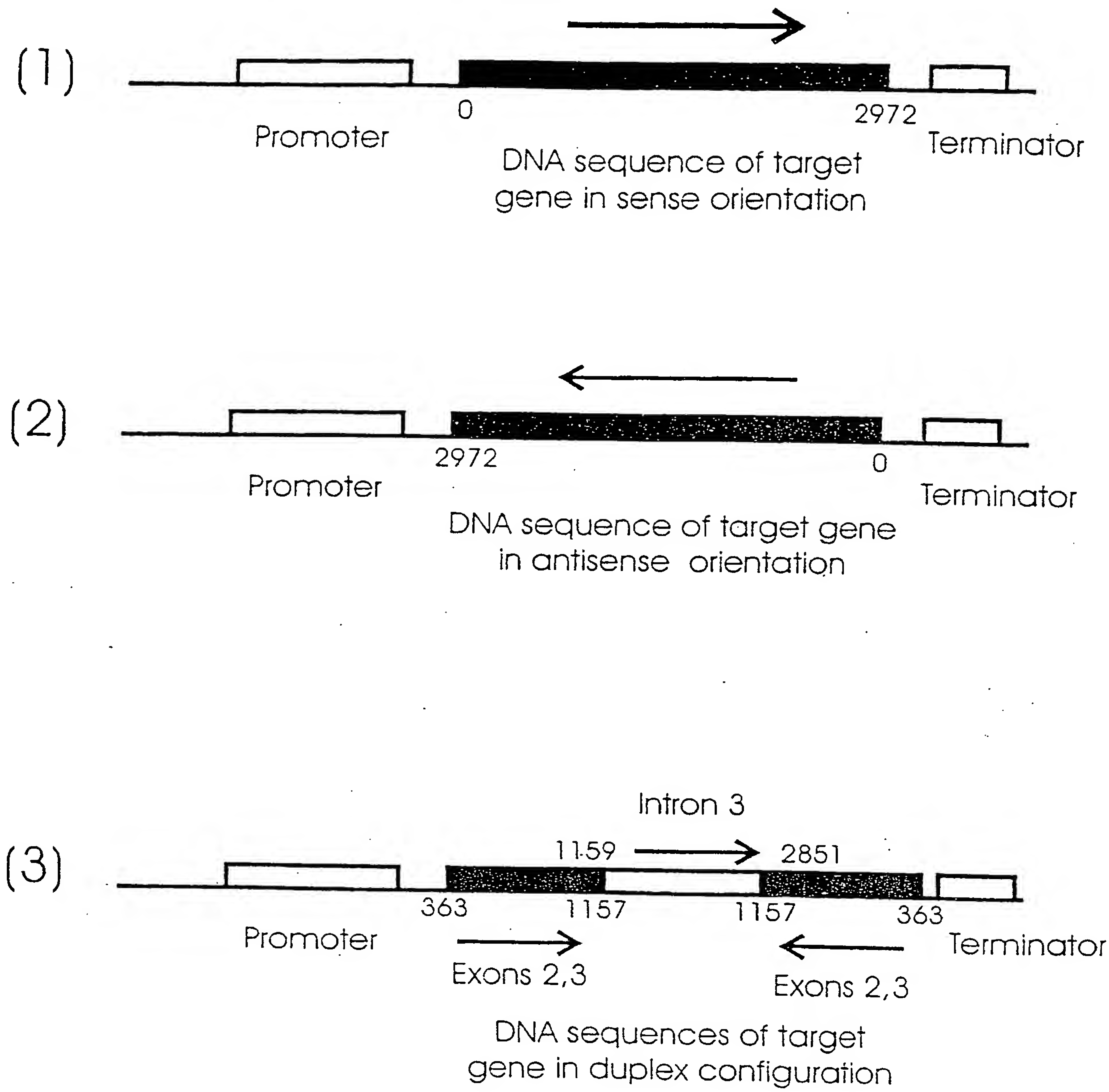

(1)
Promoter
0
2972
Terminator
DNA sequence of target gene in sense orientation
(2)
Promoter
2972
0
Terminator
DNA sequence of target gene in antisense orientation
(3)
Intron 3
1159
2851
363
1157
1157
363
Promoter
Terminator
Exons 2,3
Exons 2,3
DNA sequences of target gene in duplex configuration

FIGURE 15

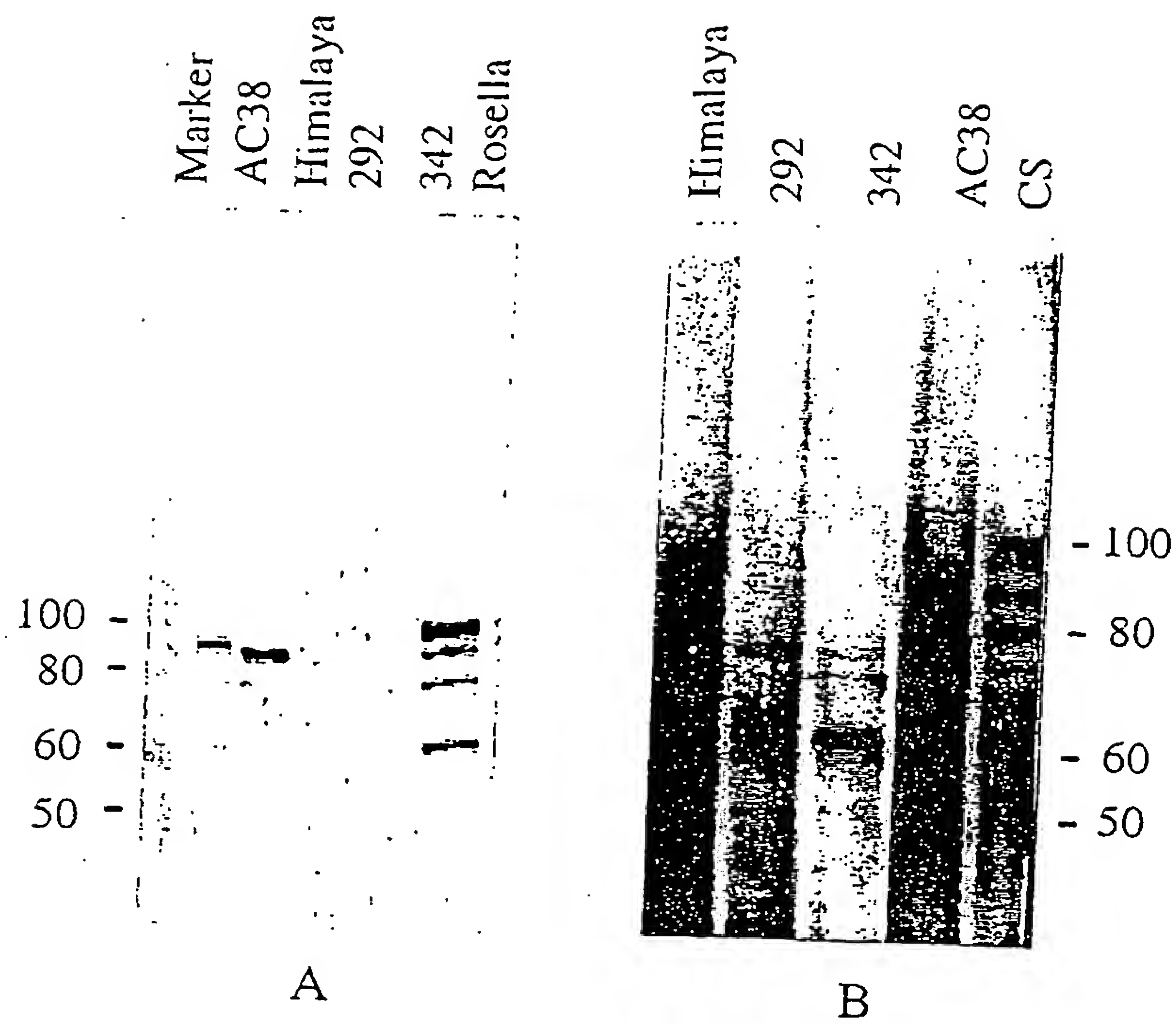

Marker
AC38
Himalaya
292
342
Rosella
100
80
60
50
A
Himalaya
292
342
AC38
CS
100
80
60
50
B

FIGURE 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/01452

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. 7: A01H 5/00, 5/10; C12N 15/29; C08L 3/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC (WPIDS) AND CHEMICAL ABSTRACTS - KEYWORDS BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
SEE BELOW

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
GENBANK, EMBL, WPIDS, CA, MEDLINE, AGRICOLA. Keywords: starch, synthase, II, 2, synthaseII, synthase2, ssII, ss2, ss, barley, hordeum, wheat, rice, maize, high()amylose, resistant()starch

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	National Plant Germplasm System (http://www.ars-grin.gov/npgs/), GRIN System accession no. GSHO 2476, 23 June 1997	1-139,143
X	WO A 00/66745 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION ET AL.) 9 November 2000	1-143
X	EMBL abstract accession no. AF155217, Li et al., 7 September 1999, "Triticum aestivum starch synthase IIA mRNA, complete cds."	140-141

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 January 2002	21 JAN 2002

Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No (02) 6285 3929	Chris Luton Telephone No : (02) 6283 2256

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU01/01452

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
WO	200066745	AU	200040924

END OF ANNEX